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DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371

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U.S. Application No.  
Unassigned  
Priority Date Claimed  
3 December 1997

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International Application. No. PCT/FR98/02615  
International Filing Date 3 December 1998

Title of Invention  
**ERYTHROVIRUS AND ITS APPLICATIONS**



Applicants For DO/EO/US

**Quang Tri NGUYEN, Antoine GARBARG-CHENON and Veronique AUGUSTE**

Applicants herewith submit to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
  - b. [X] has been transmitted by the International Bureau.
  - c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [X] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
  - a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
  - b. [ ] have been transmitted by the International Bureau.
  - c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
  - d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventors (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

**Items 11. to 14. below concern other document(s) or information included:**

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment.
- [X] Other items or information:
  - a. [X] WIPO Publication WO99/28439 (first page)
  - b. [X] PCT/IB/304
  - c. [X] PCT/IB/308
  - d. [X] Sequence Listing (paper copy only)

1.5 CLASSIFICATION NO.

INTERNATIONAL APPLICATION NO.

ATTORNEY DOCKET NUMBER

Unassigned

PCT/FR98/02615

045636-5033

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5. [X] The following fees are submitted:

**Basic National Fee (37 CFR 1.492(a)(1)-(5)):**

Search Report has been prepared by the EPO or JPO.....\$840.00

International preliminary examination fee paid to

USPTO (37 CFR 1.482).....\$670.00

No international preliminary examination fee paid to

USPTO (37 CFR 1.482) but international search fee  
paid to USPTO (37 CFR 1.445(a)(2)).....\$760.00

Neither international preliminary examination fee

(37 CFR 1.482) nor international search fee  
(37 CFR 1.445(a)(2)) paid to USPTO.....\$970.00

International preliminary examination fee paid to USPTO

(37 CFR 1.482) and all claims satisfied provisions

of PCT Article 33(2)-(4).....\$96.00

**ENTER APPROPRIATE BASIC FEE AMOUNT = \$ 840.00**

Surcharge of \$130.00 for furnishing the oath or declaration later than

[ ] 20 [ ] 30 months from the earliest claimed priority date

(37 CFR 1.492(e)).

\$

Claims	Number Filed	Number Extra	Rate	
Total Claims	34 - 20 =	14	X \$18.00	\$252.00
Independent Claims	5 - 3 =	2	X \$78.00	\$156.00
Multiple dependent claim(s) (if applicable)			+ \$260.00	\$

**TOTAL OF ABOVE CALCULATIONS = \$1248.00**

Reduction by ½ for filing by small entity, if applicable. Verified

Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28)

-\$

**SUBTOTAL = \$1248.00**

Processing fee of \$130.00 for furnishing the English translation later

than [ ] 20 [ ] 30 months from the earliest claimed priority date

(37 CFR 1.492(f)).

+\$

**TOTAL NATIONAL FEE = \$1248.00**

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The

assignment must be accompanied by an appropriate cover sheet

(37 CFR 3.28, 3.31). \$40.00 per property

+\$

**TOTAL FEES ENCLOSED = \$1248.00**

Amount to be

refunded

\$

charged

\$

- a. [ ] A check in the amount of \_\_\_\_\_ to cover the above fees is enclosed.
- b. [X] Please charge my Deposit Account No. 50-0310 in the amount of **\$1248.00** to cover the above fees. A duplicate copy of this sheet is enclosed.
- c. [X] **Except** for issue fees payable under 37 C.F.R. §1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this application including fees due under 37 CFR §1.16 and §1.17 which may be required, or credit any overpayment to Deposit Account No. 50-0310.

Customer No. 009629

SEND ALL CORRESPONDENCE TO:

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Submitted: June 2, 2000

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of : Quang Tri NGUYEN, et al.	)	
	)	
U.S. National Phase Application	)	
Filed : June 2, 2000	)	
	)	
U.S. Application No.: To Be Assigned	)	Group Art Unit: Unassigned
	)	
Date of National	)	
Stage Entry : Concurrently	)	Examiner: Unassigned
	)	
Based on PCT/FR98/02615	)	
Filed : December 3, 1998	)	
	)	
For: ERYTHROVIRUS AND ITS	)	
APPLICATIONS	)	

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

**PRELIMINARY AMENDMENT**

Prior to the examination of the above-identified application on the merits, please amend the application, without prejudice, as follows:

**IN THE CLAIMS:**

Claim 1, lines 1 and 2, change "Nucleic acids sequence, characterized in that it is" to--A nucleic acid comprising nucleic acids--

Claim 2, line 1, change, "Nucleic sequence according to" to --The nucleic acids of--;

Claim 2, line 2, change “characterized in that it has” to --wherein the nucleic acids exhibits--

Claim 3, line 1, change “sequence SEQ ID NO:1,” to --the nucleic acids--;

Claim 3, line 11, change “the fragments of” to --wherein the fragments comprise--;

Claim 4, lines 1 and 2, change “Fragment according to Claim 3, characterized in that it is” to

--A fragment according to Claim 3,--;

Claim 4, line 6, change “in that it” to --wherein the selected sequence--;

Claim 5, lines 1 and 2, change “Fragment according to Claim 3, characterized in that it is” to

--A fragment according to Claim 3,--;

Claim 5, line 7 change “in that it is” to --wherein the selected sequence--;

Claim 6, line 1, change “Pairs” to ---A pair--;

Claim 7, line 1, change “Variant” to --A variant--;

Claim 8, line 1, change “Plasmid,” to -- A plasmid--;

Claim 9, line 1, change “Plasmid” to --A plasmid--;

Claim 10, line 1, change “Diagnostic” to --A diagnostic--;

Claim 10, line 4, insert “and” before “the”;

Claim 10, lines 6 and 7, delete “, optionally labelled with an appropriate marker”;

Claim 11, line 1, change “Method” to --A method--;

Claim 12, line 1, change “Method” to --The method--;

Claim 13, line 1, change “Method” to --The method--;

Claim 13, lines 3, delete “according to Claim 6” and add:

--selected from the group consisting of:

pair A: primers SEQ ID NO:111 and SEQ ID NO:112;

pair B: primers SEQ ID NO:105 and SEQ ID NO:106;

pair C: one of the sequences SEQ ID NO:2-44, 105, 106, 107, 109, 111 or 112 and one of the sequences SEQ ID NO:45-80, 108 or 110;

pair D: primer SEQ ID NO:107 and primer SEQ ID NO:109;

pair E: two primers selected from the sequences SEQ ID NO:2-44, 105, 106, 107, 109, 111 or 112; and

pair F: two primers selected from the sequences SEQ ID NO:45-80, 108 or 110.--

Claim 14, line 1, change "Method" to --A method--;

Claim 15, please cancel the entire claim;

Claim 16, line 1, change "Method" to --A method--;

Claim 16, lines 5 and 6, please delete "optionally labeled";

Claim 17, line 1, change "Products" to --A product--;

Claim 17, line 2, change "they are" to --it is--;

Claim 18, line 1, change "Protein" to --A protein--;

Claim 19, line 1, change "Protein or peptide, characterized in that it is" to --A protein or peptide,--;

Claim 19, line 3, delete the phrase, "in that it is";

Claim 20, line 1, change "Immunogenic compositions" to --An immunogenic composition--

Claim 20, lines 3 and 4, delete “and/or one of the peptides or proteins according to Claim 18 or Claim 19”;

Claim 21, line 1, change “Antibodies” to --An antibody--;

Claim 21, lines 2 and 3, change “any one of Claims 17 to 20” to --Claim 17--;

Claim 22, please delete in its entirety;

Claim 23, please delete in its entirety;

Please add the following new claims, 24-37:

24. A method of *in vitro* screening diagnosis of infection of an individual with an erythrovirus comprising detecting hybridization of the individual’s nucleic acid with a nucleic acid according to Claim 1.

25. The method of claim 24 comprising gene amplification.

26. The method of claim 16 wherein the probe is labeled.

27. The method of claim 16 wherein the nucleic acid of the virus to be typed is labeled.

28. An immunogenic composition comprising one or more of the proteins of claim 18.

29. An immunogenic composition comprising one or more of the peptides or proteins of claim 19.

30. An antibody directed against one or more of the proteins of claim 18.

31. An antibody directed against one or more of the proteins of claim 19.

32. A method for the immunological *in vitro* screening diagnosis of infection of an individual with an erythrovirus comprising detecting anti-erythrovirus V9 antibodies by contacting a biological sample with a peptide according to claim 17 and detecting the association of such a peptide with antibodies contained in the biological sample by an appropriate means.

33. The method of claim 32 wherein the appropriate detection means is selected from the group consisting of EIA, ELISA, RIA, and fluorescence.

34. A method for the immunological *in vitro* screening diagnosis of infection of an individual with an erythrovirus comprising detecting erythrovirus V9 viral proteins by contacting a biological sample with an antibody according to claim 21 and detecting the association of such an antibody with erythrovirus V9 viral proteins contained in the biological sample by an appropriate means.

35. The method of claim 34 wherein the appropriate detection means is selected from the group consisting of EIA, ELISA, RIA, and fluorescence.

36. An erythrovirus diagnostic kit comprising at least one probe of sequence SEQ ID NO: 45-80, 108 or 110, and/or a pair of primers selected from the group consisting of , and/or a peptide encoded by, or capable of being expressed with the aid of a nucleic acid of Claim 1, and/or an antibody directed against such peptides.

37. The diagnostic reagent of claim 10 wherein the reagent is labeled with an appropriate marker.

**REMARKS**

The changes to the claims requested above have been made so as to eliminate multiple claim dependencies and to present claim language more conventional for practice in the United States. These changes do not introduce new matter, nor do they alter the subject matter presented and examined in the corresponding International Application.

Respectfully submitted,

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ERYTHROVIRUS AND ITS APPLICATIONS

The present invention relates to nucleic sequences derived from a human erythrovirus, to their fragments as well as to their applications as diagnostic reagent and as an immunogenic agent.

Sero-epidemiological studies show that infection with the parvovirus B19, recently renamed erythrovirus B19, is commonly and widely distributed worldwide.

In Europe, the seroprevalence for erythrovirus B19 is about 10% in subjects under 5 years, about 50% for subjects over 20 years and greater than 90% in elderly persons.

The high seroprevalence rate suggests that erythrovirus B19 is highly contagious. During epidemics, the rate of transmission to subjects in close contact is 10 to 60%, the route of transmission being mainly aerial (respiratory secretions).

Erythrovirus B19 is a specifically human virus. Acute infection commonly causes benign maculopapular skin rashes in children (epidermal megalerythema or 5<sup>th</sup> disease). Arthralgias may accompany the rashes and may exceptionally become chronic.

A transient acute erythroblastic attack usually occurs in patients already carrying a chronic haemolytic anaemia (sickle cell anaemia, thalassaemia, pyruvate kinase deficiency and the like), causing a transient aregenerative acute anaemia.

Acute primary infection with erythrovirus B19 is particularly dangerous in pregnant women with a risk of transmission to the foetus estimated at 30%. The risk of foetal death by anaemia, hepatic insufficiency, cardiac insufficiency and foetoplacental anasarca is estimated at between 5 and 9%.

Chronic infections with erythrovirus B19 are found essentially in immunosuppressed subjects (chronic myeloid leukaemia, humoral and cellular immune

deficiency, organ or marrow transplants, AIDS diseases).

In seropositive HIV-1 patients, chronic infection with erythrovirus B19 is responsible for chronic anaemia, but can also act on the other lineages (neutropenia and especially thrombopenia). The absence of a sufficient humoral immune response in these patients allows the installation of a chronic erythroviraemia and explains both the chronic erythroblastopenia and the absence of other symptoms such as rash or arthralgias.

Erythrovirus B19 is a virus having a single-stranded DNA genome of about 5.4 kbases; it is the only erythrovirus classified to date; all the strains which have been sequenced and which have been the subject of a publication in the sequence libraries (GenBank or EMBL) exhibit a low genetic variability (98% nucleic sequence similarity over the whole genome and 96% similarity over the VP1 region) (R.O. SHADE, *J. Virol.*, 1986, 58, 3, 921-936, B19-AU).

Virological diagnosis of erythrovirus B19 infections is based essentially on the detection of the viral genome, insofar as the culture cannot be carried out routinely.

For acute infections with erythrovirus B19 (primary infections), this detection can be made by gene amplification (PCR), but also by hybridization (dot-blot) given the viral titre, which is usually very high during primary infections (up to  $10^{14}$ /ml of serum); however, the viral titre is much lower during chronic infections and only a gene amplification detection method can be envisaged.

These detection techniques are dependent on the genetic variability of the virus tested for; the reagents prepared from known erythrovirus B19 sequences do not make it possible to detect the variant erythrovirus infections, either by gene amplification or by B19 serodiagnosis.

Indeed, the existing serodiagnostic tests are specific for erythrovirus B19 (International Application PCT WO 91/12269; International Application PCT WO 96/09391 (IDEIA<sup>®</sup> Parvovirus B19 IgG and IgM, DAKO; Parvovirus B19 IgG and IgM *Enzyme Immunoassay*, BIOTRIN)).

Consequently, the detection techniques specified above risk producing negative results both at the nucleic level and with respect to the antibody response.

The identification and the taking into account of new variants are important for developing:

- reagents for the detection and diagnosis of human erythrovirus infections (serodiagnosis, PCR, hybridization), which are sufficiently sensitive and specific, that is to say which do not lead to false-negative or false-positive results,
- compositions capable of protecting against all erythrovirus infections (vaccines), and
- compositions capable of treating a variant erythrovirus infection (serotherapy, monoclonal antibodies).

The inventors therefore set themselves the aim of providing erythrovirus-derived sequences capable of allowing the detection of a variant erythrovirus (called erythrovirus type V9), that is to say which is genetically distant from erythrovirus B19.

The subject of the present invention is a nucleic acid sequence, characterized in that it is selected from the group consisting of:

- the sequences derived from an erythrovirus which, molecularly, cannot be recognized as an erythrovirus B19 because it exhibits a genetic divergence or distance  $\geq 10\%$  ( $< 90\%$  similarity) over the whole genome with respect to the erythrovirus B19 sequences and which exhibit a genetic divergence of less than or equal to  $6\%$  ( $> 94\%$  similarity) with respect to the sequence SEQ ID NO:1,
- the sequence SEQ ID NO:1, and

- the nucleotide sequences capable of hybridizing under stringent conditions with the said sequence ID NO:1.

5 This variant erythrovirus is called type V9 variant.

Stringent conditions are understood to mean, for the purposes of the present invention, the following conditions:

10 . hybridization for 3 to 24 h in a 1XSSC buffer containing 50% formamide, at 42°C, and

. 3 washes of 15 min in a 2XSSC buffer, at 60°C.

15 The sequence SEQ ID NO:1, which corresponds to about 95% of the genome of an erythrovirus type V9 and which includes all the coding sequences, has a restriction map which is different from that of the B19 erythroviruses, in particular as regards the BamHI site (no site), HINDIII site (only one site) and PvuII site (five sites).

20 More precisely, the sequence SEQ ID NO:1 has a restriction profile which is different from that of erythrovirus B19, in particular by the following restriction sites: AccI, AflIII, AlwI, AlwNI, **ApaI**,  
AvaI, **AvaII**, **AvrII**, **BamHI**, BanI, BanII, BbeI, BbsI,  
25 BceFI, BcgI, BcnI, **BglII**, BsgI, BsiEI, BsmI, BsmAI, Bsp120I, BspHI, BspMI, BsrFI, Bst1107I, BstEII, BstUI, Bsu36I, DpnI, DraIII, DsaI, EaeI, EagI, EarI, Ec1136I, EcoNI, Eco109I, EcoRI, EheI, FokI, HaeI, HaeIII, HgaI, HgiAI, HhaI, HincII, **HindIII**, HinPI, HpaI, KasI, MaeII,  
30 MboI, McrI, MscI, **MunI**, NarI, NciI, NcoI, NsiI, NspI, Nsp7524I, NspBII, NspCI, PflMI, PmeI, Ppu10I, PpuMI, PstI, **PvuII**, SacI, Sau3AI, ScaI, SfaNI, SfcI, SmaI, SpeI, SphI, SspI, StuI, StyI, SwaI, Tth111I, XbaI, XmaI and their isoschizomers.

35 The subject of the present invention is also fragments of sequence ID NO:1 which are capable of allowing the detection of an erythrovirus V9 and characterized in that they comprise a nucleotide sequence selected from the group consisting of:

- a) a sequence corresponding to positions 328-2340 of SEQ ID NO:1, encoding the NS1 protein (SEQ ID NO:81),  
b) a sequence corresponding to positions 1796-2017 of SEQ ID NO:1, encoding the 7.5 kDa protein (SEQ ID NO:83),  
5 c) a sequence corresponding to positions 2336-4678 of SEQ ID NO:1, encoding the VP1 protein (SEQ ID NO:85),  
d) a sequence corresponding to positions 2336-3016 of SEQ ID NO:1, encoding the VP1u (SEQ ID NO:87),  
10 e) a sequence corresponding to positions 2523-2828 of SEQ ID NO:1, encoding the X protein (SEQ ID NO:89),  
f) a sequence corresponding to positions 3017-4678 of SEQ ID NO:1, encoding the VP2 (SEQ ID NO:91),  
g) a sequence corresponding to positions 4488-4883 of SEQ ID NO:1, encoding the 11 kDa protein (SEQ ID:93),  
15 h) a nucleotide sequence capable of hybridizing with one of the sequences SEQ ID NO:1, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91 or SEQ ID NO:93,  
20 i) the sequences SEQ ID NO:2-80,  
j) the sequences SEQ ID NO:105 (E1905f), 106 (E1987r), 107 (E2076f), 108 (E2151r), 109 (E2406r), 110 (E2149rs), 111 (E2717f), 112 (E2901r), 113 (e1855f), 114 (e2960r), 115 (e1863f), 116 (e2953), 117 (e2435fStul/BglII), 118 (e4813rEcoRI), 119 (e3115fBamHI), 120 (e4813rBamHI) and 121 (e1954fp) and  
25 k) the sequences complementary to the preceding sequences, the fragments derived from the preceding sequences of at least 17 nucleotides or their  
30 complementary sequences.

For the purposes of the present invention, nucleic sequence or nucleotide sequence (DNA or RNA sequence) is understood to mean one of the sequences as defined above and their complementary sequences (anti-sense sequences) as well as the sequences comprising  
35 one or more of the said sequences or fragments thereof.

The invention also includes nucleotide fragments complementary to the preceding ones as well as fragments modified with respect to the preceding

ones by removal or addition of nucleotides in a proportion of about 15%, with respect to the length of the above fragments and/or modified at the level of the nature of the nucleotides, as long as the modified  
5 nucleotide fragments retain a capacity for hybridization with the erythrovirus V9 DNA or RNA sequence which is similar to that exhibited by the corresponding unmodified fragments.

Some of these fragments are specific and are  
10 used as a probe or primer; they hybridize specifically to an erythrovirus V9 or a related erythrovirus; a virus related to erythrovirus V9 is understood to mean an erythrovirus exhibiting a genetic divergence of less than or equal to 6%; these fragments are selected from  
15 the group consisting of the sequences SEQ ID NO:45-80 and NO:108 and 110, or their complementary sequences, the sequences derived from these sequences of at least 17 nucleotides and the sequences comprising the said sequences and they find application in the specific  
20 identification of an erythrovirus V9 or of a related erythrovirus.

Others of these fragments are used as primers, for the amplification of sequences derived from an erythrovirus type V9 or a related virus, such as the  
25 sequence SEQ ID NO:1; these primers are chosen from the group consisting of the sequences SEQ ID NO:2-44 and the sequences SEQ ID NO:105-109 and 111-121 or their complementary sequences and the sequences derived from the said sequences, of at least 17 nucleotides.

30 The said fragments also include, in the case of primers, the antisense sequences.

Such sequences find application for the differential identification of erythroviruses (erythrovirus B19 and erythrovirus V9), combined with  
35 probes as defined above and/or with suitable restriction enzymes.

The said primers preferably comprise between 17 and 30 nucleotides; preferred primers are the following: the sequence SEQ ID NO:105 (positions 1797-

1815 of the sequence SEQ ID NO:1), which corresponds to the sequence SEQ ID NO:10, the sequence SEQ ID NO:106 (positions 1899-1879 of the sequence SEQ ID NO:1), which corresponds to a fragment of the antisense sequence of the sequence SEQ ID NO:11, the sequence SEQ ID NO:107 (positions 1968-1987 of the sequence SEQ ID NO:1), which corresponds to a fragment of the sequence SEQ ID NO:13, the sequence SEQ ID NO:108 (positions 2061-2043 of the sequence SEQ ID NO:1), which corresponds to a fragment of the antisense sequence of the sequence SEQ ID NO:58, the sequence SEQ ID NO:109 (positions 2317-2298 of the sequence SEQ ID NO:1), which corresponds to a fragment of the antisense sequence of the sequence SEQ ID NO:16, the sequence SEQ ID NO:111 (positions 2609-2627 of the sequence SEQ ID NO:1), which corresponds to a fragment of the sequence SEQ ID NO:19 and the sequence SEQ ID NO:112 (positions 2812-2793 of the sequence SEQ ID NO:1), which corresponds to a fragment of the antisense sequence of the sequence SEQ ID NO:23.

Preferred pairs of primers are the following:

- pair A: primers SEQ ID NO:111 and SEQ ID NO:112;
- pair B: primers SEQ ID NO:105 and SEQ ID NO:106;
- pair C: one of the sequences SEQ ID NO:2-44, 105, 106, 107, 109, 111 or 112 and one of the sequences SEQ ID NO:45-80, 108 or 110;
- pair D: primer SEQ ID NO:107 and primer SEQ ID NO:109;
- pair E: two primers selected from the sequences SEQ ID NO:2-44, 105, 106, 107, 109, 111 or 112;
- pair F: two primers selected from the sequences SEQ ID NO:45-80, 108 or 110.

These various primers can be used, depending on the fragment amplified, as sense primer or as antisense primer.

The subject of the present invention is also a variant erythrovirus, characterized in that its genome cannot be recognized molecularly as an erythrovirus B19, in that it exhibits a divergence of less than or equal to 6% with the sequence SEQ ID NO:1, as defined above, and in that its genome hybridizes specifically, under stringent conditions, as defined above, with one of the sequences SEQ ID NO:45 to 80, 108 and 110, as defined above.

The subject of the present invention is also a plasmid, characterized in that it comprises the viral genome of a variant erythrovirus strain, called erythrovirus V9 or a fragment thereof, which cannot be recognized molecularly as an erythrovirus B19 and which exhibits with the latter a genetic divergence of  $\geq 10\%$  over the whole genome with respect to the erythrovirus B19 sequences and a divergence of less than or equal to 6% with the sequence SEQ ID NO:1.

The viral genome of the said erythrovirus V9 is considered to be genetically distant from erythrovirus B19.

According to an advantageous embodiment of the said plasmid, it includes the sequence SEQ ID NO:1 (PCD.V9.C22).

The subject of the present invention is also a diagnostic reagent for the differential detection of type V9 erythroviruses, characterized in that it is selected from the sequences SEQ ID NO:45-80, 108 and 110, optionally labelled with an appropriate marker.

Among the appropriate markers, there may be mentioned radioactive isotopes, enzymes, fluorochromes, chemical markers (biotin and the like), haptens (digoxigenin and the like) and antibodies or appropriate base analogues.

The subject of the present invention is also a process for the rapid and differential detection of erythroviruses, by hybridization and/or gene amplification, using a biological sample as starting



material, which process is characterized in that it comprises:

(1) a step in which a biological sample to be analysed is brought into contact with at least one  
5 probe of sequence SEQ ID NO:45-80, 108 or 110, and

(2) a step in which the product(s) resulting from the erythrovirus nucleotide sequence-probe interaction is (are) detected by any appropriate means.

Preferably, the hybridization comprises a pre-  
10 hybridization which is carried out in a buffer which comprises 5-60% of formamide; 1-5X SSC; 2% of blocking reagent (*Blocking buffer*, Boehringer Mannheim, Meylan, France); 0.1% of N-laurylsarcosine; 0.01-5% of SDS, at  
15 carried out in 3 ml of a buffer of the same composition with 10 µl of labelled probe at 40-70°C for 1-30 hours.

In accordance with the said process, it may comprise, prior to step (1):

. a step extracting the nucleic acid to be  
20 detected, belonging to the virus genome, which may be present in the biological sample, and

. at least one gene amplification cycle.

The gene amplification step is in particular carried out with the aid of one of the following gene  
25 amplification techniques: amplification with Q $\beta$ -replicase (I. Haruna et al., Proc. Nat. Acad. Sci. USA, 1965, 54, 579-587), PCR (polymerase chain reaction) (R.K. Saiki et al., 1986, Nature, 324:163-6), LCR (ligase chain reaction) (F. Barany, Proc. Nat.  
30 Acad. Sci. USA, 1991, 88, 189-193), ERA (end-run amplification) (C. Adams et al., 1994, Novel amplification technologies for DNA/RNA-based diagnostics meeting, San Francisco, CA, United States),  
CPR (cycling probe reaction) (P. Duck et al., Bio-  
35 techniques, 1990, 9, 142-147) or SDA (strand displacement amplification) (GT. Walker, 1994, SDA: novel amplification technologies for DNA/RNA-based diagnostics meeting, San Francisco, CA, United States).

According to an advantageous embodiment of the said process, the amplification cycles are carried out with the aid of a pair of primers selected from the sequences SEQ ID NO:2-44, 105-109 and 111-112 and  
5 fragments of these sequences, preferably from the pairs of primers as defined above.

When pair A is used, the amplification product is advantageously screened by the action of the restriction enzyme ApaI (GGGCCC): the product of  
10 amplification of a B19 genome is cleaved with ApaI (generating 2 fragments of 149 and 55 base pairs (bp)) whereas the product of amplification of a V9 genome is not cleaved by ApaI (a fragment of 204 bp); an agarose or acrylamide gel electrophoresis makes it possible to  
15 distinguish between these restriction fragments.

When pair B is used, the product of amplification is advantageously screened by the action of one of the following restriction enzymes: BglII (AGATCT), or MunI (CAATTG); different fragments are  
20 thus obtained depending on whether an erythrovirus V9 or B19 is involved; a fragment which comprises a BglII restriction site is specific for the variant erythrovirus V9 as defined above, whereas the B19 erythroviruses comprise an MunI site in this region.  
25 The product of amplification of a B19 genome is cleaved with MunI (generating 2 fragments of 36 and 67 bp) and is not cleaved by BglII (a fragment of 103 bp) whereas the product of amplification of a V9 genome is cleaved by BglII (2 fragments of 19 and 84 bp) and is not  
30 cleaved by MunI (a fragment of 103 bp); an agarose or acrylamide gel electrophoresis makes it possible to distinguish between these different restriction fragments.

When pair C is used (a primer capable of  
35 hybridizing with all erythroviruses and a primer capable of specifically hybridizing with erythrovirus V9) or when pair F is used (two primers capable of specifically hybridizing with erythrovirus V9), the V9

genome is amplified whereas there is no specific amplification with the B19 genome.

When pair D is used, the product of amplification is advantageously screened by hybridization with a labelled specific probe for erythrovirus V9, selected from the sequences SEQ ID NO:58-60 and 110, preferably by hybridization with the probe of sequence SEQ ID NO:110; the product of amplification of a V9 genome hybridizes specifically with these probes and in particular the probe of sequence SEQ ID NO:110, whereas the product of amplification of a B19 genome does not hybridize with the abovementioned probes.

When pair E is used, the product of amplification is screened by any method of hybridization with a probe specific for erythrovirus V9, selected from the sequences SEQ ID NO:45-80, 108 and 110; in this case, the product of amplification of a V9 genome hybridizes with the probe, but not the product of amplification of a B19 genome.

The subject of the invention is also the use of the sequences described above, of fragments derived from these sequences or of their complementary sequences, for carrying out a method of hybridization or of gene amplification of erythrovirus nucleic sequences, these methods being applicable to the *in vitro* diagnosis of the potential infection of an individual with an erythrovirus type V9.

The subject of the present invention is also a method of screening and typing an erythrovirus V9 or a related virus, characterized in that it comprises bringing a probe selected from the group consisting of the sequences SEQ ID NO:45-80, 108 and 110, optionally labelled, into contact with the nucleic acid of the virus to be typed and detecting the nucleic acid-probe hybrid obtained.

The subject of the present invention is also products of translation, characterized in that they are encoded by a nucleotide sequence as defined above.

The subject of the present invention is also a protein, characterized in that it is in particular capable of being expressed with the aid of a nucleotide sequence selected from the group consisting of the sequences SEQ ID NO:81, 83, 85, 87, 89, 91 and 93, as defined above and the derived peptides comprising between 7 and 50 amino acids.

Peptide is understood to mean below both the proteins and the peptides, as defined above.

Such peptides are in particular capable of being recognized by antibodies induced by an erythrovirus V9 and/or of inducing the production of anti-erythrovirus V9 antibodies.

The said peptides are in particular selected from the sequences SEQ ID NO:82 (NS1 protein), SEQ ID NO:86 (VP1 protein), SEQ ID NO:88 (single VP1 protein), SEQ ID NO:92 (VP2 protein) and SEQ ID NO:95-104, namely fragments of the VP1 protein [VP1a peptide (SEQ ID NO:95); VP1b peptide (SEQ ID NO:96); VP1c peptide (SEQ ID NO:97); peptide VP1d (SEQ ID NO:98); peptide VP1e (SEQ ID NO:99) and peptide VP1f (SEQ ID NO:100)], or fragments of the VP2 protein [peptide VP2a (SEQ ID NO:101); peptide VP2b (SEQ ID NO:102); peptide VP2c (SEQ ID NO:103); peptide VP2d (SEQ ID NO:104)] as well as the derived peptides comprising 7 to 50 amino acids.

The subject of the invention is also immunogenic compositions comprising one or more products of translation of the nucleotide sequences according to the invention and/or one or the peptides as defined above, obtained in particular by synthetic means.

The subject of the invention is also the antibodies directed against one or more of the peptides described above and their use for carrying out in particular a differential in vitro method of diagnosis of the infection of an individual with an erythrovirus.

The subject of the present invention is also a method for the immunological detection of an erythrovirus V9 infection, characterized in that it comprises:

- for the detection of anti-erythrovirus V9 antibodies, bringing a biological sample into contact with a peptide according to the invention (serodiagnosis),

- 5       - for the detection of erythrovirus V9 viral proteins, bringing a biological sample into contact with an antibody according to the invention;

the reading of the result being revealed by an appropriate means, in particular EIA, ELISA, RIA, 10 fluorescence.

By way of illustration, such an in vitro method of diagnosis according to the invention comprises bringing a biological sample, collected from a patient, into contact with antibodies according to the invention 15 or peptides according to the invention, and detecting, with the aid of any appropriate method, in particular with the aid of labelled anti-immunoglobulins, immunological complexes formed between the antigens or the antibodies of the erythroviruses which may be 20 present in the biological sample and the said antibodies or the said peptides, respectively.

The reagents according to the invention are in particular useful for the detection of the V9 erythroviruses and related viruses in pregnant women, in HIV- 25 positive patients with anaemia and/or chronic thrombopenia, recipients of organ or marrow transplants, and patients having a central acute anaemia and for whom the tests for the detection of erythrovirus B19 are negative.

30       The subject of the invention is, in addition, an erythrovirus diagnostic kit, characterized in that it includes at least one reagent according to the invention (probes, pairs of primers, peptides or antibodies).

35       In addition to the preceding features, the invention further comprises other features which will emerge from the description which follows, which refers to exemplary embodiments of the method which is the

subject of the present invention as well as to the appended drawings, in which:

- Figures 1, 2 and 3 illustrate phylogenetic trees for erythrovirus V9: Figure 1: phylogenetic tree for the complete erythrovirus sequence; Figure 2: phylogenetic tree for the erythrovirus NS1 genes; Figure 3: phylogenetic tree for the erythrovirus VP1 genes;
- Figures 4, 5 and 6 represent the genetic distances for the complete erythrovirus sequences (Figure 4), for the erythrovirus NS1 genes (Figure 5) and for the erythrovirus VP1 genes (Figure 6);
- Figure 7 illustrates the restriction map of sequence ID NO:1.

It should be understood, however, that these examples are given solely by way of illustration of the subject of the invention and do not constitute in any manner a limitation thereto.

**EXAMPLE 1: Production of sequences conforming to the invention**

An AatII/AatII restriction fragment of 5028 bp, representing virtually the entire (95%) genome of the V9 variant, was cloned into the sequencing vector pcDNA2.1 (Invitrogen, Netherlands) in the following manner.

The single-stranded viral DNA was extracted from the serum of a patient with an acute erythroblastopenic attack with the aid of a QIAamp Blood Kit column (Qiagen S.A., France). Using a step of hybridization in a 50 mM NaCl buffer at 56°C for 16 hours, the viral DNA is converted to double-stranded DNA. Next, 1.3 µg of double-stranded viral DNA is subjected to the AatII restriction enzyme (18 U) at 37°C for 2 hours, the restriction enzyme is then inactivated at 65°C for 15 minutes. The product is dialysed on a Millipore VSWPO13000 cellulose acetate and nitrate membrane against water for 2 hours. The double-stranded viral DNA AatII/AatII restriction

fragment thus prepared is frozen at -20°C while awaiting the ligation step.

The vector pcDNA2.1 is modified in order to receive the AatII fragment by site-directed insertion mutation: the EagI restriction site of the multiple cloning site was removed and replaced with an AatII site. The vector pcDNA2.1a thus produced was amplified in bacterial culture and purified with the aid of a QIAfilter Plasmid Maxi Kit (Qiagen S.A., France). Next, 3 µg of the vector pcDNA2.1a is subjected to restriction with the enzyme AatII at 37°C for 1 hour and then dephosphorylated with shrimp alkaline phosphatase (Boehringer Mannheim, Meylan, France). The enzymes are inactivated at 65°C for 15 minutes.

The ligation is carried out with a vector/viral DNA insert molar ratio of 1/1, that is to say 50 ng of vector and 100 ng of viral DNA insert, prepared as described above, with the aid of 1 U of T4 ligase (Life Technologies, France) at 24°C for 16 hours. After a 1/2 dilution, the ligation product is heated at 65°C in order to inactivate the T4 ligase and then cooled on ice. Electrocompetent bacteria Sure<sup>®</sup> (Stratagene, Heidelberg, Germany) are electrophorized with 2 or 4 µl of this ligation solution (1500 V, 50 µF, 200 Ω) and then incubated with 1 ml of SOC medium (Life Technologies, France) for 1 hour before being spread on a Luria Broth agar medium (Life Technologies, France) containing 100 µg/ml of amoxycillin, 15 µg/ml of tetracycline, 100 µg/ml of IPTG and 50 µg/ml of X-gal.

Twenty four (recombinant) white colonies were selected, their plasmid is extracted by minipreparation of DNA and a rough restriction map (AatII, AatII + BamHI, BamHI, BamHI + BglII, HindII) made it possible to select 2 recombinant clones with an insert having a size and a restriction map compatible with a V9 viral DNA insert.

These 2 clones (2 and 22) were sequenced with the aid of an automated sequencer ABI 377 (Perkin Elmer, France): they indeed contain an insert of

5028 bp, the 2 sequences are identified except at position 1165 (A and G for the clones 2 and 22 respectively). The direct sequence of the V9 viral DNA made it possible to determine that it is the G at position 1165 which is correct; it is therefore clone 22 which was selected (PCD.V9.C22), whose sequence corresponds to SEQ ID NO:1.

Figures 1 to 6 show the genetic distances which exist between erythrovirus V9 and erythrovirus B19. In these figures, the different erythrovirus sequences are represented by their mnemonic in GenBank (release 103.0 of October 1997).

**EXAMPLE 2: Diagnosis of an erythrovirus type V9 by DNA hybridization (dot blot or slot blot or microplate) with a specific probe**

The viral DNA is extracted, for example, with the aid of a QIAamp Blood Kit column (Qiagen S.A., France) or of any other method of extracting nucleic acids from a biological sample (blood, serum, plasma, amniotic fluid, bone marrow, tissue). The DNA in solution is denatured at 95°C for 2 minutes and then cooled on ice, transferred onto nylon or cellulose nitrate membrane by vacuum filtration and then fixed (heating of the membrane at 80°C for 1 hour). The membrane is then hybridized under stringent conditions with a DNA or RNA probe specific for V9, such as the sequence SEQ ID NO:1 or its complementary sequence or a fragment thereof, in particular the sequences SEQ ID NO:45 to SEQ ID NO:80 and 110 and their complementary sequences, or a fragment of these sequences which are appropriately labelled. This labelling may be a labelling with a radioelement ( $^{32}\text{P}$ ,  $^{33}\text{P}$ ,  $^{35}\text{S}$ ,  $^3\text{H}$ ,  $^{14}\text{C}$  or another radio isotope), a cold labelling (biotin), fluorescent marker, digoxigenin or any other molecule which may be coupled or incorporated into a DNA or RNA fragment and which can be detected by a specific antibody, or by a ruthenium chelate). In the case of a labelling with a radioactive element, the visualization is performed by autoradiography or any other method



allowing the detection of the radioisotope emission (such as Phosphorimager, Molecular Dynamics, Bondoufle, France). In the case of a labelling with biotin, the visualization is performed with the aid of an enzyme/streptavidine conjugate and a suitable visualization substrate. In the case of a fluorescent labelling, the visualization is made with the aid of a fluoro-Imager (Molecular Dynamics, Bondoufle, France) or any other apparatus capable of detecting the fluorescence emission. In the case of a labelling with digoxigenin (or with another antigen), the visualization is made with the aid of an anti-digoxigenin antibody (or an antibody specific for the antigen used for the labelling), coupled directly to an enzyme (alkaline phosphatase, peroxidase or any other enzyme), or in an indirect manner with an anti-digoxigenin antibody (or an antibody specific for the antigen used for the labelling) and an antibody coupled to an enzyme. A substrate suitable for the enzyme of the conjugate is used for the visualization. In the case of a labelling with ruthenium chelate (such as TBR), the visualization is performed by an electrochemiluminescence reaction (G.F. Blackburn et al., Clin. Chem., 1991, 37:1534-1539).

A variant of this technique comprises the fixing of viral DNA on a microplate or another solid support and hybridization with a labelled probe as specified above.

Another variant of this technique comprises the fixing of an unlabelled probe on a microplate or another solid support and hybridization with the viral DNA of the sample which would have been labelled beforehand.

**EXAMPLE 3:** Diagnosis of an erythrovirus type V9 by gene amplification (PCR or polymerase chain reaction) and hybridization

Viral DNA is extracted from a biological sample (blood, serum, plasma, amniotic fluid, bone marrow, tissue) with the aid of a QIAamp Blood Kit column

(Qiagen S.A., France) or of any other method of extracting nucleic acids.

The PCR is carried out according to the method described by Saiki et al. (Nature, 1986, 324: 163-66) with 10 µl of DNA solution in a final volume of 100 µl of reaction mixture (50 mM KCl; 10 mM Tris-HCl pH 8.3; 2.5 mM MgCl<sub>2</sub>; 200 µM dNTP; 25 pmol of sense and antisense oligonucleotides) with 1.5 IU of AmpliTaq Gold™ (Perkin Elmer, France). The amplification primers are oligonucleotides of 20 to 25 mers chosen so as to amplify only the DNA of the V9 variant: either the 2 primers (sense and antisense) are fragments of the sequences specific for V9 (SEQ ID NO:45 to 80, 108 and 110) or their complementary sequences, or one of the primers is chosen from the sequences specific for V9 (SEQ ID NO:45-80, 108 and 110) or their complementary sequences whereas the other primer is chosen from the sequences capable of hybridizing both the B19 erythroviruses and the V9 erythroviruses (SEQ ID NO:2 to 44, 105-107, 109 and 111-112) or their complementary sequences. The temperature cycles are applied to the reaction mixture by a thermocycler (T9600, Perkin Elmer, France) according to the following programme:

1 cycle:

- 6 minutes at 95°C

5 cycles:

- 60 seconds at 95°C

- 30 seconds at 60°C

- 30 seconds at 72°C

45 cycles:

- 30 seconds at 95°C

- 30 seconds at 60°C

- 30 seconds at 72°C

1 cycle:

- 5 minutes at 72°C

The product of amplification is deposited on a 1.3% agarose gel so as to be subjected to an electrophoretic separation and a transfer onto a nylon membrane loaded by capillarity according to a

conventional technique (Sambrook J. et al., 1989, *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor).

5 The probe is an oligonucleotide of 20-30 mers, a fragment of a sequence specific for V9 (SEQ ID NO:45 to 80, 108 and 110) or their complementary sequences. It is labelled in 3' with DIG-dUTP with the aid of the DIG Oligonucleotide Tailing kit (Boehringer Mannheim, Meylan, France).

10 The transfer membrane is prehybridized in a buffer comprising (50% formamide; 5X SSC; 2% of blocking reagent (Boehringer Mannheim, Meylan, France); 0.1% of N-laurylsarcosine; 0.02% of SDS), at 42°C for 90 minutes. The hybridization is carried out in 3 ml of  
15 a buffer of the same composition with 10 µl of labelled probe at 42°C for 16 hours. The membrane is washed twice in 2X SSC buffer containing 0.1% SDS at 60°C for 10 minutes, and then twice in 1X SSC buffer containing 0.1% SDS at 60°C for 10 minutes. The membrane is then  
20 visualized with DIG Luminescent Detection Kit (Boehringer Mannheim, Meylan, France) and an autoradiography.

**EXAMPLE 4:** Group diagnosis and differential diagnosis of type B19 and V9 erythroviruses by gene amplification  
25 and hybridization

The viral DNA is extracted from a biological sample (blood, serum, plasma, amniotic fluid, bone marrow, tissue) with the aid of a QIAamp Blood Kit column (Qiagen S.A., France) or of any other method of  
30 extracting nucleic acids.

The PCR is carried out according to the method described by Saiki et al. (Nature, 1986, cited above) with 10 µl of DNA solution in a final volume of 100 µl of reaction mixture (50 mM KCl; 10 mM Tris-HCl pH 8.3;  
35 2.5 mM MgCl<sub>2</sub>; 200 µM dNTP; 25 pmol of sense and antisense oligonucleotides) with 1.5 IU of AmpliTaq Gold™ (Perkin Elmer, France). The amplification primers are oligonucleotides of 20 to 25 mers chosen so as to amplify the DNA of B19 and of the variant V9: the 2

(sense and antisense) primers are fragments of the sequences capable of hybridizing both with the B19 erythroviruses and with the V9 erythroviruses (SEQ ID NO:2 to 44, 105-107, 109, 111-112) or of their  
5 complementary sequences. The temperature cycles are applied to the reaction mixture by a thermocycler (T9600, Perkin Elmer, France) according to the following programme:

- 1 cycle:
  - 10 - 6 minutes at 95°C
- 5 cycles:
  - 60 seconds at 95°C
  - 30 seconds at 60°C
  - 30 seconds at 72°C
- 15 45 cycles:
  - 30 seconds at 95°C
  - 30 seconds at 60°C
  - 30 seconds at 72°C
- 1 cycle:
  - 20 - 5 minutes at 72°C

The product of amplification is deposited on a 1.3% agarose gel so as to be subjected to an electrophoretic separation and a transfer onto a nylon membrane loaded by capillarity according to a  
25 conventional technique (Sambrook J. et al., 1989, cited above).

The probe is an oligonucleotide of 20-30 mers, a fragment of a sequence specific for V9 (SEQ ID NO:45 to 80, 108 and 110) or their complementary sequences,  
30 or alternatively specific for B19, or finally which hybridizes both with B19 and with V9 (SEQ ID NO:2 to 44 or 105-107, 109, 111-112), if it is sought to carry out a group diagnosis. It is labelled in 3' with DIG-dUTP with the aid of the DIG *Oligonucleotide Tailing kit*  
35 (Boehringer Mannheim, Meylan, France).

The transfer membrane is prehybridized and hybridized under the same conditions as those set out in Example 3.

**EXAMPLE 5: Group diagnosis and differential diagnosis of type B19 and V9 erythroviruses by gene amplification and restriction enzymes**

Extraction of the viral DNA from a biological sample (blood, serum, plasma, amniotic fluid, bone marrow, tissue) with the aid of a QIAamp Blood Kit column (Qiagen S.A., France) or of any other method of extracting nucleic acids.

The NS1a PCR is carried out according to the method described by Saiki et al. with 5 µl of DNA solution in a final volume of 50 µl of reaction mixture (50 mM KCl; 10 mM Tris-HCl pH 8.3; 2.5 mM MgCl<sub>2</sub>; 200 µM dNTP; 12.5 pmol of sense and antisense oligonucleotides) with 1.5 IU of AmpliTaq Gold™ (Perkin Elmer, France) and the pair of primer B (sense primer e1905f, SEQ ID NO:105; and the antisense primer e1987r, SEQ ID NO:106) using the following temperature cycles (on a thermocycler T9700, Perkin Elmer, France):

- 1 cycle:
  - 6 minutes at 94°C
- 5 cycles:
  - 30 seconds at 94°C
  - 1 minute at 55°C
  - 1 minute at 72°C
- 45 cycles:
  - 30 seconds at 94°C
  - 30 seconds at 60°C
  - 30 seconds at 72°C
- 1 cycle:
  - 7 minutes at 72°C

An aliquot of the product of amplification (10 µl) was deposited on a 2% agarose gel so as to be subjected to an electrophoretic separation and a transfer onto a nylon membrane loaded by capillarity according to a conventional technique (J. Sambrook et al., 1989, cited above). The membrane was hybridized with an oligonucleotide probe of 36 mer, e1954fp (SEQ ID NO:121): ACCAGTATCAGCAGCAGTGGTGGTGAAAGCTCTGAA, a

fragment of the sequence SEQ ID NO:11. This probe allows a detection of type B19 and V9 erythroviruses.

5 An aliquot of the product of amplification (10 µl) was subjected to the action of the restriction enzyme MunI for 2 hours and then subjected to an electrophoretic separation on a 2% agarose gel. As described above, the erythrovirus type is B19 if there is cleavage, and V9 if there is no cleavage.

Results of the NS1a PCR:

10 79 samples found to be indeterminate or weakly positive with the old B19 PCR (Lefrere, et al., Transfusion, 1995, 35:389-391) were screened with the aid of the new NS1a PCR (consensus erythrovirus, sequences according to the invention). Of the 79  
15 samples screened, 31 are positive and were typed with the aid of the restriction enzyme MunI: 18 (58%) were found to be of type B19 and 13 (42%) of type V9.

The samples which were positive by NS1a PCR were able to be amplified on 1100 bp by a nested PCR  
20 (S1S2 PCR) with the aid of the pair of primers e1855f (SEQ ID NO:113) and e2960r (SEQ ID NO:114) for the first amplification step of 30 cycles (PCRS1), and of the pair of primers e1863f (SEQ ID NO:115) and e2953r (SEQ ID NO:116) for the second amplification step of 50  
25 cycles (PCRS2). 15 samples were found to be positive by S1S2 PCR and sequenced on 1110 bp (13 of type B19 by NS1A PCR and 2 of the variant type). The analysis of the sequences showed that:

- the B primers (sense primer e1905f, SEQ ID  
30 NO:105; and antisense primer e1987r, SEQ ID NO:106), are perfectly conserved for all the 15 sequences (of the B19 and variant type) as well as for all the known B19 sequences, confirming their importance for use for a consensus diagnostic test for B19 and V9,  
35 - the probe e1954fp (SEQ ID NO:121), a fragment of the sequence SEQ ID NO:11 is equally well conserved for the 15 sequences as well as for all the known B19 sequences,

- the B19 sequences form a well homogeneous group with less than 1.2% divergence between them (7 B19 sequences of GenBank and the 13 B19 sequences of this study),

- 5        - finally for the 2 sequences typed variant erythrovirus by NS1a PCR with MunI digestion, less than 4.5% divergence with V9 is observed.

EXAMPLE 6: Cloning of the capsid genes VP1 and VP2 of V9 into a baculovirus expression vector

- 10        First step:

- cloning of the VP1 gene into a bacterial plasmid

The VP1 gene of V9 is amplified by PCR according to the method described by Saiki et al.

- 15        (Nature, 1986, 324:163-166) with 10 µl of a 10<sup>-2</sup> dilution of V9 viral DNA in a final volume of 100 µl of reaction mixture (20 mM Tris-HCl pH 8.8; 10 mM KCl, 10 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>; 2 mM MgSO<sub>4</sub>; 0.1% Triton X-100; 0.1 mg/ml of BSA; 0.2 mM dNTP; 25 pmol of sense primers (e2435fStuI/BglIII: AAAGGCCTAGATCTTGTAGATTATGAGTAAAAC, SEQ ID NO:117) and antisense primers (e4813rEcoRI: GGGAAATTCGGTGGGTGACGGTTCCTG, SEQ ID NO:118) with 2.5 U of Pfu Turbo™ (Stratagene, France). The amplification primers were chosen on the V9 sequence on either side
- 20        of the VP1 gene, their 5' end was modified by addition of restriction site(s) (indicated in their name) in order to facilitate the cloning. The temperature cycles applied to the reaction mixture are the following:

1 cycle:

- 30        - 1 minute at 94°C

20 cycles:

- 1 minute at 94°C

- 1 minute at 55°C

- 2.5 minutes at 72°C

- 35        1 cycle:

- 10 minutes at 72°C

The product of amplification of the VP1 gene was purified with the aid of a silica column (QIAquick PCR Purification Kit, Qiagen, France) and then

subjected to the action of the restriction enzymes StuI and EcoRI. After heat inactivation of the restriction enzymes (20 min at 65°C), the VP1 gene fragment was purified by dialysis against H<sub>2</sub>O on a 0.025 µm filter

5 (VSWP01300, Millipore).

The plasmid pBacPAK8 (Clontech, France) is subjected to the action of the restriction enzymes StuI and EcoRI, the vector is then dephosphorylated with shrimp alkaline phosphatase (Boehringer, France). After

10 heat inactivation of the restriction enzymes (20 min at 65°C), the plasmid was purified with the QIAquick PCR Purification Kit (Qiagen).

The ligation is carried out with 50 ng of plasmid pBacPAK8 and 100 ng of VP1 fragment (prepared

15 as described above) with T4 ligase (Life Technologies, France). After heat inactivation of the T4 ligase (10 min at 65°C), 2 µl of ligation product diluted 1/2 with water are electroporated with 25 µl of electro-

20 competent bacteria (Epicurian Coli Sure Electroporation-Competent cells, Strategene). The electroporated bacteria are immediately taken up in 1 ml of SOC medium (2% tryptone, 0.5% of yeast extracts, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl<sub>2</sub>, 10 mM MgSO<sub>4</sub> and 20 mM glucose), incubated for 1 h at 37°C,

25 with stirring. Next, 10 µl, 100 µl and 890 µl of the transformed bacteria are plated on Lennox agar dishes (10 g/l of peptone, 5 g/l of yeast extracts, 5 g/l NaCl, and 13 g/l agar) containing 50 µg/ml of ampicillin. After 24 h of incubation at 37°C, 24

30 colonies per construct are subcultured in 5 ml of Lennox medium with 50 µg/ml of ampicillin and incubated for 24 h at 37°C, with stirring.

The plasmid DNA is extracted by alkaline minilysis with the aid of the QIAprep 8 Turbo miniprep

35 kit (Qiagen) and analysed by StuI/EcoRI and KpnI/-HindIII restriction in order to determine the presence of the insert and its orientation. The clone pB8-VP1.C5 was selected and the recombinant plasmid was checked by sequencing.



- cloning of the VP2 gene into a bacterial plasmid.

The VP2 gene of V9 is amplified by PCR according to the method described by Saiki et al. (Nature, 1986, 324:163-166) with 10  $\mu$ l of a  $10^{-2}$  dilution of V9 viral DNA in a final volume of 100  $\mu$ l of reaction mixture (20 mM Tris-HCl pH 8.8; 10 mM KCl, 10 mM  $(\text{NH}_4)_2\text{SO}_4$ ; 2 mM  $\text{MgSO}_4$ ; 0.1% Triton X-100; 0.1 mg/ml of BSA; 0.2 mM dNTP; 25 pmol of sense primers (e3115fBamHI: CACGGATCCATACCCCGAGCATGACTTCAG, SEQ ID NO:119) and antisense primers (e4813rBamHI: CACGGATCCGGTGGGTGACGGTTCCTG, SEQ ID NO:120) with 2.5 U of Pfu Turbo<sup>TM</sup> (Stratagene, France). The amplification primers were chosen on the V9 sequence on either side of the VP2 gene, their 5' end was modified by addition of restriction site(s) (indicated in their name) in order to facilitate the cloning. The temperature cycles applied to the reaction mixture are the following:

- 1 cycle:
  - 1 minute at 94°C
- 20 cycles:
  - 1 minute at 94°C
  - 1 minute at 60°C
  - 2.5 minutes at 72°C
- 1 cycle:
  - 10 minutes at 72°C

The product of amplification of the VP2 gene was purified with the aid of a silica column (QIAquick PCR Purification Kit, Qiagen, France) and then subjected to the action of the restriction enzymes BamHI. The VP2 gene fragment was purified by the QIAquick PCR Purification Kit (Qiagen).

The plasmid pBacPAK8 (Clontech, France) is subjected to the action of the restriction enzymes BamHI, the vector is then dephosphorylated with shrimp alkaline phosphatase (Boehringer, France). After heat inactivation of the shrimp alkaline phosphatase (20 min at 65°C), the plasmid was purified by phenol/chloroform extraction and precipitated with ethanol.

The ligation is carried out with 50 ng of plasmid pBacPAK8 and 100 ng of VP2 fragment (prepared as described above) with T4 ligase (Life Technologies, France). After heat inactivation of the T4 ligase (10 min at 65°C), 2 µl of ligation product diluted 1/2 with water are electroporated with 25 µl of electro-competent bacteria (Epicurian Coli Sure Electroporation-Competent cells, Stratagene). The electroporated bacteria are immediately taken up in 1 ml of SOC medium, incubated for 1 h at 37°C, with stirring. Next, 10 µl, 100 µl and 890 µl of the transformed bacteria are plated on Lennox agar dishes containing 50 µg/ml of ampicillin. After 24 h of incubation at 37°C, 24 colonies per construct are sub-cultured in 5 ml of Lennox medium with 50 µg/ml of ampicillin and incubated for 24 h at 37°C, with stirring.

The plasmid DNA is extracted by alkaline minilysis with the aid of the QIAprep 8 Turbo miniprep kit (Qiagen) and analysed by BamHI and SacI restriction in order to determine the presence of the insert and its orientation. The clone pB8-VP2.C20 was selected and the recombinant plasmid was checked by sequencing: a base A deleted just upstream of the initiator ATG of VP2 can be noted, but this mutation can be ignored: it will not generate the expression of VP2.

Second step:

- Construction of the recombinant baculovirus expressing VP1

The plasmid pB8-VP1.C5 is cotransfected with the baculovirus BacPAK6, linearized with Bsu361 (BacPAK™ Baculovirus Expression System, Clontech), into SF9 insect cells with lipofectin. 2 isolations are performed by the lysis plaque method, the plaques isolated are transferred onto a nitrocellulose membrane, the membrane is then hybridized with a DNA probe specific for the VP1 gene of V9.

The recombinant baculovirus BacPAK6-pB8-VP1.C4.2 was thus selected. The expression of the VP1

protein was verified by Western Blotting on a cellular pellet of SF9 cells infected with this recombinant baculovirus. A band was observed at the expected size of VP1 (about 80 kDa) but which is not recognized by  
5 the anti-VP1-B19 monoclonal antibody (Argène, France). It is possible that this monoclonal antibody does not crossreact with the VP1 protein of V9.

The cloning into a baculovirus was verified by sequencing after PCR with the primers Bac1 and Bac2  
10 (Clontech).

- Construction of the recombinant baculovirus expressing VP2

The plasmid pB8-VP2.C20 is cotransfected with the baculovirus BacPAK6, linearized with Bsu361  
15 (BacPAK™ Baculovirus Expression System, Clontech), into SF9 insect cells with lipofectin. 2 isolations are performed by the lysis plaque method, the plaques isolated are transferred onto a nitrocellulose membrane, the membrane is then hybridized with a DNA  
20 probe specific for the VP2 gene of V9.

The recombinant baculovirus BacPAK6-pB8-VP2.-C1.3 is selected. The expression of the VP2 protein was verified by Western Blotting on a cellular pellet of SF9 cells infected with this recombinant baculovirus.  
25 The anti-VP2-B19 monoclonal antibody (Argène, France) indeed detects a protein with an apparent molecular weight of about 58 kDa which is also clearly visible on the acrylamide gel. Virus-like particles of about 20 to 30 nm in diameter are observed by electron microscopy  
30 in the culture supernatants of the SF9 cells after infection with a recombinant baculovirus expressing the VP2 protein of V9. The size and the appearance of the virus-like particles obtained are in every respect in conformity with those described for B19. This  
35 observation confirms that the VP2 protein of V9 is produced in a native form by the baculovirus, because it is capable of forming empty capsids by self-assembling.

The cloning into a baculovirus was verified by sequencing after PCR with the primers Bac1 and Bac2 (Clontech).

Third step:

- 5           The proteins VP1 and VP2 of V9 expressed in a baculovirus will be purified so as to be used as a target antigen for new serological tests for the diagnosis of erythrovirus V9 infections.

- 10           As is evident from the above, the invention is not at all limited to its embodiments, implementations and applications which have just been described more explicitly; it encompasses on the contrary all the variants which may occur to the specialist in this field, without departing from the framework or the  
15           scope of the present invention.

CLAIMS

1. Nucleic acid sequence, characterized in that it  
5 is selected from the group consisting of:

- the sequence SEQ ID NO:1,
- the genomic sequences of variant erythro-  
viruses, called erythrovirus type V9, which,  
molecularly, cannot be recognized as an erythrovirus  
10 B19 because it exhibits a genetic divergence  $\geq 10\%$  over  
the whole genome with respect to the erythrovirus B19  
sequences and which exhibit a genetic divergence of  
less than or equal to 6% with respect to the sequence  
SEQ ID NO:1, and
- 15 - the erythrovirus genomic sequences capable of  
hybridizing under stringent conditions with one of the  
following sequences: SEQ ID NO:45-80, SEQ ID NO:81, SEQ  
ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ  
ID NO:91, SEQ ID NO:93, SEQ ID NO:108, SEQ ID NO:110,  
20 SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119 and SEQ ID  
NO:120.

2. Nucleic sequence according to Claim 1,  
characterized in that it has a restriction profile  
according to Figures 7.1 to 7.3.

25 3. Fragments of sequence SEQ ID NO:1, according to  
Claim 1, which are capable of allowing the detection of  
an erythrovirus V9, characterized in that they are  
selected from the group consisting of:

a) the sequences SEQ ID NO:81, SEQ ID NO:83,  
30 SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91  
or SEQ ID NO:93,

b) the sequences SEQ ID NO:2-80

c) the sequences SEQ ID NO:105-121, and-

d) the sequences complementary to the preceding  
35 sequences, the fragments of at least 17 nucleotides  
derived from the preceding sequences or their  
complementary sequences.

4. Fragment according to Claim 3, characterized in that it is selected from the group consisting of the sequences SEQ ID NO:45-80, 108 and NO: 110, their complementary sequences, the sequences of at least 17 nucleotides derived from these sequences and the sequences comprising the said sequences and in that it is capable of serving as a probe in the specific identification of an erythrovirus V9 or of a related erythrovirus.

5. Fragment according to Claim 3, characterized in that it is selected from the group consisting of the sequences SEQ ID NO:2-80 and the sequences SEQ ID NO:105-121, their complementary sequences, the sequences of at least 17 nucleotides derived from these sequences and the sequences comprising the said sequences and in that it is capable of serving as a primer for the amplification of sequences derived from an erythrovirus.

6. Pairs of primers, characterized in that they are selected from the group consisting of:

- pair A: primers SEQ ID NO:111 and SEQ ID NO:112;

- pair B: primers SEQ ID NO:105 and SEQ ID NO:106;

- pair C: one of the sequences SEQ ID NO:2-44, 105, 106, 107, 109, 111 or 112 and one of the sequences SEQ ID NO:45-80, 108 or 110;

- pair D: primer SEQ ID NO:107 and primer SEQ ID NO:109;

- pair E: two primers selected from the sequences SEQ ID NO:2-44, 105, 106, 107, 109, 111 or 112; and

- pair F: two primers selected from the sequences SEQ ID NO:45-80, 108 or 110.

7. Variant erythrovirus, characterized in that it cannot be recognized molecularly as an erythrovirus B19 genome, and in that it exhibits a genetic divergence of less than or equal to 6% with the sequence SEQ ID NO:1

and in that its genome hybridizes specifically, under stringent conditions with one of the sequences SEQ ID NO:45 to 80, 108 and 110.

8. Plasmid, characterized in that it comprises the viral genome of a variant erythrovirus strain, called erythrovirus V9, which cannot be recognized molecularly as an erythrovirus B19 and which exhibits with the latter a genetic divergence of  $\geq 10\%$  over the whole genome with respect to the erythrovirus B19 sequences and a genetic divergence of less than or equal to 6% with the sequence SEQ ID NO:1 or a fragment thereof, according to Claim 3.

9. Plasmid according to Claim 8, characterized in that it includes the sequence SEQ ID NO:1.

10. Diagnostic reagent for the differential detection of type V9 erythroviruses, characterized in that it is selected from the sequences SEQ ID NO:45-80, 108 and 110, their complementary sequences, the sequences of at least 17 nucleotides, derived from these sequences, optionally labelled with an appropriate marker.

11. Method for the rapid and differential diagnosis of erythroviruses, by hybridization and/or gene amplification, using a biological sample as starting material, which process is characterized in that it comprises:

(1) a step in which a biological sample to be analysed is brought into contact with at least one probe of sequence SEQ ID NO:45-80, 108 or 110, and

(2) a step in which the product(s) resulting from the erythrovirus nucleotide sequence-probe interaction is (are) detected by any appropriate means.

12. Method according to Claim 11, characterized in that it comprises, prior to step (1):

. a step of extracting of the nucleic acid to be detected, belonging to the virus genome, which may be present in the biological sample, and

. at least one gene amplification cycle.

13. Method according to Claim 12, characterized in that the amplification cycles are carried out with the aid of a pair of primers according to Claim 6.

14. Method for the rapid and differential diagnosis of erythroviruses, characterized in that it comprises:

. a step of extracting of the nucleic acid to be detected, belonging to the virus genome, which may be present in the biological sample,

. at least one gene amplification cycle with the aid of a pair of primers according to Claim 6, and

. the detection of the amplified product, on the one hand, by hybridization with the sequence SEQ ID NO:121 and, on the other hand, by the action of the restriction enzyme MunI.

15. Use of the sequences according to any one of Claims 1 to 5, for carrying out a method of hybridization or of gene amplification of erythrovirus nucleic sequences, these methods being applicable to the in vitro diagnosis of the potential infection of an individual with an erythrovirus.

16. Method of screening and typing an erythrovirus V9 or a related virus, characterized in that it comprises bringing a probe selected from the group consisting of the sequences according to Claim 4, optionally labelled, into contact with the nucleic acid of the virus to be typed, optionally labelled, and detecting the nucleic acid-probe hybrid obtained.

17. Products of translation, characterized in that they are encoded by a nucleotide sequence according to Claim 1.

18. Protein, characterized in that it is capable of being expressed with the aid of a nucleotide sequence according to Claim 1.

19. Protein or peptide, characterized in that it is derived from a variant erythrovirus type V9, as defined in Claim 1 and in that it is selected from the sequences:



a) SEQ ID NO:82 (NS1 protein), SEQ ID NO:86 (VP1 protein), SEQ ID NO:88 (single VP1 protein), SEQ ID NO:92 (VP2 protein) and SEQ ID NO:95-104, namely fragments of the VP1 protein [VP1a peptide (SEQ ID NO:95); VP1b peptide (SEQ ID NO:96); VP1c peptide (SEQ ID NO:97); peptide VP1d (SEQ ID NO:98); peptide VP1e (SEQ ID NO:99); peptide VP1f (SEQ ID NO:100)], or fragments of the VP2 protein [peptide VP2a (SEQ ID NO:101); peptide VP2b (SEQ ID NO:102); peptide VP2c (SEQ ID NO:103); peptide VP2d (SEQ ID NO:104)], and

b) the sequences derived from sequences a) comprising between 7 and 50 amino acids.

20. Immunogenic compositions comprising one or more products of translation of the nucleotide sequences according to Claim 17 and/or one of the peptides or proteins according to Claim 18 or Claim 19.

21. Antibodies directed against one or more of the peptides or proteins according to any one of Claims 17 to 20.

22. Method for the immunological detection of an erythrovirus V9 infection, characterized in that it comprises:

- for the detection of anti-erythrovirus V9 antibodies, bringing a biological sample into contact with a peptide according to any one of Claims 17 to 19 (serodiagnosis),

- for the detection of erythrovirus V9 viral proteins, bringing a biological sample into contact with an antibody according to Claim 21;

the reading of the result being revealed by an appropriate means, in particular EIA, ELISA, RIA, fluorescence.

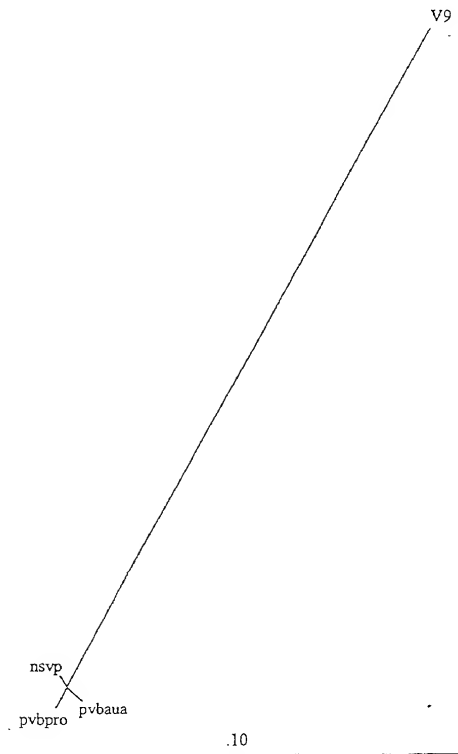
23. Erythrovirus diagnostic kit, characterized in that it includes at least one reagent according to Claim 11 and/or a pair of primers according to Claim 6 and/or a peptide according to any one of Claims 17 to 19 and/or an antibody according to Claim 21.

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FIGURE 1

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V9

pvl19x528  
pvl19x599  
e09420  
pvl19nsvp  
pvlbpro  
pvl19x560  
pvlbaua

0.10

FIGURE 2

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vq

cbu38507

cbu38506

ebu38518  
ebu38509-

cbu38209-

pvb19x528

ebu38515

cbu389  
bny313

est

сбыва

Αριθμός 09420

Doc 78508 pvb19x599

chu68543.c

COLE 8546

cbu38513

51799424 pvl9nsvp

92560

38511 PVG19350  
38514 PVG19350

514 8510

0.10

FIGURE 3

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	nsvp	aua	pro	v9
nsvp	0.00	0.70	0.80	14.77
pvbaua		0.00	0.90	15.03
pvbpro			0.00	15.03
v9				0.00

FIGURE 4

	420	599	nsvp	560	aua	528	pro	V9
e09420	0.00	0.65	0.75	0.70	0.80	0.75	0.85	14.87
pvb19x599		0.00	0.70	0.65	0.75	0.70	0.80	14.93
pvb19xnsvp			0.00	0.35	0.45	0.70	0.70	14.99
pvb19x560				0.00	0.40	0.65	0.65	14.98
pvbaua					0.00	0.75	0.75	15.06
pvb19x528						0.00	0.80	15.17
pvbpro							0.00	15.11
v9								0.00

FIGURE 5

FIGURE 6

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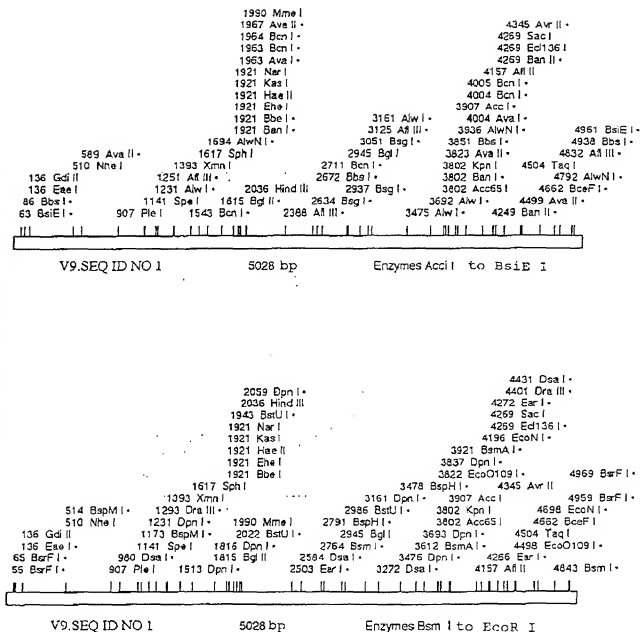


FIGURE 7.1

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2241 HnPI-  
 2100 FokI-  
 2036 HindIII-  
 1990 MmeI  
 1964 NciI-  
 1963 NciI-  
 1944 HnPI-  
 1944 HhaI-  
 1922 HnPI-  
 1922 HhaI-  
 1921 NarI-  
 1921 KsaI-  
 1921 HaeII  
 1921 EheI-  
 1921 BbeI-  
 1640 FokI-  
 1617 SphI-  
 1543 NciI-  
 1440 FokI-  
 1310 HaeIII-  
 1247 HincII-  
 1231 MboI-  
 1141 SpeI-  
 980 NcoI-  
 907 PstI-  
 695 FokI-  
 2754 HaeIII-  
 2730 HaeIII-  
 2711 NciI-  
 2388 MaeII-  
 2283 HpaI-  
 2241 HhaI-  
 2206 FokI-  
 2059 MboI-  
 2023 HgaI-  
 1815 MboI-  
 1815 BglII  
 2283 HincII-  
 2085 HincII-  
 3238 HgaI-  
 3161 MboI-  
 3026 HpaI-  
 3026 HincII-  
 2953 HaeIII-  
 2810 MunI-  
 3476 MboI-  
 3457 MaeII-  
 3125 MaeII-  
 2945 BglI  
 4431 NcoI-  
 4413 MunI-  
 4410 HaeIII-  
 4345 AvrII  
 4269 SacI  
 4008 HaeIII-  
 3907 AccI  
 3888 HaeIII-  
 4157 AflII  
 4005 NciI-  
 3802 KpnI  
 3837 MboI-  
 3802 Acc65I  
 3693 MboI-  
 4269 HgaI-  
 4269 Ecd136I  
 4004 NciI-  
 3659 HgaI-  
 4504 TaqI  
 5024 MaeII-  
 5009 MaeII-  
 4961 MorI-  
 4667 HgaI-  
 4662 BceFI  
 4269 HgaI-  
 4269 Ecd136I  
 4794 HgaI-  
 4504 TaqI

V9.SEQ ID NO 1

5028 bp

Enzymes Ehe I to Nco I

2059 Sau3AI-  
 1990 MmeI  
 1963 SmaI-  
 1921 NarI  
 1921 KsaI  
 1921 HaeII  
 1921 EheI  
 1816 Sau3AI-  
 1815 BglII  
 1617 SphI-  
 1617 NspCI-  
 1393 XmnI  
 1363 PstII-  
 1318 ScaI-  
 1315 PstII-  
 1251 NspCI-  
 1251 Nsp7524I-  
 937 PstII-  
 810 NheI  
 219 PvuII-  
 219 NspBII-  
 136 GdiII  
 136 EaeI  
 72 SfaNI-  
 1601 SfaNI-  
 2036 HindIII  
 1231 Sau3AI-  
 1141 SpeI-  
 1921 BbeI  
 1617 Nsp7524I-  
 1617 NspI-  
 1513 Sau3AI-  
 4400 PstII-  
 4361 SspI-  
 4345 AvrII  
 4289 PvuII-  
 4289 NspBII-  
 4269 SacI  
 4269 Ecd136I  
 4179 NspCI-  
 3965 ScaI-  
 3748 SclI-  
 3729 PvuII-  
 3720 NspBII-  
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 3365 SfaNI-  
 3169 SfaNI-  
 3161 Sau3AI-  
 3084 NspCI-  
 3084 Nsp7524I-  
 3033 SclI-  
 3033 PstI-  
 2945 BglI  
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 2940 NspBII-  
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 4004 SmaI-  
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 3907 AccI  
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 3822 PpuMI-  
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V9.SEQ ID NO 1

5028 bp

Enzymes NciI to Ssp I

FIGURE 7.2



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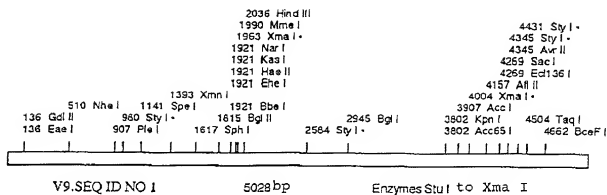


FIGURE 7.3

# COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

U.S. DEPARTMENT OF COMMERCE  
Patent and Trademark Office

ATTORNEY DOCKET NO.: 045636-5033  
APPLICATION SERIAL NO:

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

## ERYTHROVIRUS AND ITS APPLICATIONS

the specification of which:

is attached hereto; or

was filed as United States application Serial No. \_\_\_\_\_ on June 2, 2000 and was amended on \_\_\_\_\_ (if applicable); or

was filed as PCT international application Number PCT/FR98/02615 on December 3, 1998 and was amended under PCT Article 19 on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office information which is material to the patentability of claims presented in this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate or §365(a) of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

### PRIOR FOREIGN APPLICATION(S):

COUNTRY (if PCT, indicate PCT)	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
FRANCE	FR 97 15197	3 December 1997	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

**Combined Declaration For Patent Application and Power of Attorney - (Continued)**  
(includes Reference to PCT International Applications)

ATTORNEY DOCKET NO.: 045636-5033

I hereby claim the benefits under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

**U.S. PROVISIONAL APPLICATIONS**

U.S. PROVISIONAL APPLICATION NO.

U.S. FILING DATE



I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or §365(c) of any PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to the patentability of claims presented in this application in accordance with Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

**PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT:**

**U.S. APPLICATIONS**

**STATUS (Check One)**

U.S. APPLICATION NO.	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

**POWER OF ATTORNEY:** As a named inventor, I hereby appoint the registered practitioners of Morgan, Lewis & Bockius LLP included in the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and direct that all correspondence be addressed to that Customer Number.

**Customer Number:** 009629

Direct Telephone Calls To:  
(name and telephone number)

**Reid G. Adler**  
**202-467-7756**

Combined Declaration For Patent Application and Power of Attorney - (Continued)  
(includes Reference to PCT International Applications)

ATTORNEY DOCKET NO.: 045636-5033

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.



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POST OFFICE ADDRESS	129, avenue Maurice Thorez, F-94200 Ivry sur Seine, France	
FIRST OR SOLE INVENTOR'S SIGNATURE		DATE <u>17 JUL. 2000</u>
FULL NAME OF SECOND INVENTOR	<u>Antoine GARBARG-CHENON</u>	
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POST OFFICE ADDRESS	8, place Charles Fillion, F-75017 Paris, France	
SECOND INVENTOR'S SIGNATURE		DATE <u>17 JUL. 2000</u>
FULL NAME OF THIRD INVENTOR	<u>Véronique AUGUSTE</u>	
RESIDENCE & CITIZENSHIP	22/30, rue du Borrego, F-75020 Paris, France <u>FR</u>	COUNTRY OF CITIZENSHIP FRANCE
POST OFFICE ADDRESS	22/30, rue du Borrego, F-75020 Paris, France	
THIRD INVENTOR'S SIGNATURE		DATE <u>17 JUL. 2000</u>

Listing of Inventors Continued on attached page(s) [ ] Yes [X] No

## LISTAGE DES SEQUENCES

<110> NGUYEN, Quang Tri  
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 AUGUSTE, Véronique  
 ASSISTANCE PUBLIQUE-HOPITAUX DE PARIS

<120> ERYTHROVIRUS HUMAIN, FRAGMENTS DUDIT VIRUS AINSI QUE LEURS APPLICATIONS

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 <151> 1997-12-03

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&lt;211&gt; 103

&lt;212&gt; DNA

&lt;213&gt; erythrovirus

&lt;400&gt; 44

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ggcgaccggc ggcatctgat ttgggtgtctt ctttttgaaa ttt 103

&lt;210&gt; 45

&lt;211&gt; 210

&lt;212&gt; DNA

&lt;213&gt; erythrovirus

&lt;400&gt; 45

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tatataagca gctgcgttcc ctagacattt cttttctggt tgcttttgac tggaaactcac 180

ttgctgttct ttgcctgcta agtaacagg 210

&lt;210&gt; 46

&lt;211&gt; 30

&lt;212&gt; DNA

&lt;213&gt; erythrovirus

&lt;400&gt; 46

atttatacta acttttaatt tactaacatg 30

&lt;210&gt; 47

&lt;211&gt; 100

&lt;212&gt; DNA

&lt;213&gt; erythrovirus

&lt;400&gt; 47

gagctatttc ggggtgtctt gcacatttcc tctaaccattc tggactgtgc taatgataac 60

tgggtggtgc ctatgctaga cttagatact tctgactggg 100

&lt;210&gt; 48

&lt;211&gt; 117

&lt;212&gt; DNA

&lt;213&gt; erythrovirus

&lt;400&gt; 48

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<400> 61

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 tgccgcccag attatatgtg cccctccaa taccocgtag gcaaccatct ataaaagata 120  
 cagacgtgtg agaataataa ttattaacta gatatgaaca acatgtaatt agaattgtaa 180  
 gattatgtaa tatgtacaca agtttgga aataaaagcc ttaataaat aattcatagt 240  
 gtatgggttct ttaaaaattt 260  
  
 <210> 81  
 <211> 2013  
 <212> DNA

## &lt;213&gt; erythrovirus

&lt;400&gt; 81

atg gag cta ttt cgg ggt gtc ttg cac att tcc tct aac att ctg gac	48
Met Glu Leu Phe Arg Gly Val Leu His Ile Ser Ser Asn Ile Leu Asp	
1 5 10 15	
tgt gct aat gat aac tgg tgg tgc tct atg cta gac tta gat act tct	96
Cys Ala Asn Asp Asn Trp Trp Cys Ser Met Leu Asp Leu Asp Thr Ser	
20 25 30	
gac tgg gaa cca cta acc cat tct aac aga tta atg gca ata tat tta	144
Asp Trp Glu Pro Leu Thr His Ser Asn Arg Leu Met Ala Ile Tyr Leu	
35 40 45	
agc agt gtt gct tct aaa ctt gat ttt act ggg ggg ccg cta gca ggt	192
Ser Ser Val Ala Ser Lys Leu Asp Phe Thr Gly Gly Pro Leu Ala Gly	
50 55 60	
tgc tta tac ttt ttt cag gtg gaa tgt aac aaa ttt gag gaa ggc tat	240
Cys Leu Tyr Phe Phe Gln Val Glu Cys Asn Lys Phe Glu Glu Gly Tyr	
65 70 75 80	
cat atc cat gta gtt att ggt ggt cca gga cta aat gct aga aac tta	288
His Ile His Val Val Ile Gly Gly Pro Gly Leu Asn Ala Arg Asn Leu	
85 90 95	
act gtg tgc gta gaa ggt tta ttt aat aat gtt ctt tac cat ctt gta	336
Thr Val Cys Val Glu Gly Leu Phe Asn Asn Val Leu Tyr His Leu Val	
100 105 110	
act gaa agt gtt aaa ctt aaa ttt ttg cca ggg atg act acc aaa gga	384
Thr Glu Ser Val Lys Leu Lys Phe Leu Pro Gly Met Thr Thr Lys Gly	
115 120 125	
aaa tat ttt aga gat gga gag cag ttt ata gaa aat tac tta atg aaa	432
Lys Tyr Phe Arg Asp Gly Glu Gln Phe Ile Glu Asn Tyr Leu Met Lys	
130 135 140	
aaa att cct tta aat gtt gtg tgg tgt gta aca aat att gac ggg tat	480
Lys Ile Pro Leu Asn Val Val Trp Cys Val Thr Asn Ile Asp Gly Tyr	
145 150 155 160	
ata gac acc tgt att tcc gcc tct ttt cgg cga gga gct tgt cat gct	528
Ile Asp Thr Cys Ile Ser Ala Ser Phe Arg Arg Gly Ala Cys His Ala	
165 170 175	
aaa aga ccc cgc att act gca aat aca gac agt gct act aat gaa act	576
Lys Arg Pro Arg Ile Thr Ala Asn Thr Asp Ser Ala Thr Asn Glu Thr	
180 185 190	
ggg gag tct agc tgt gga ggg gga gat gtt gtg cca ttc gct gga aag	624
Gly Glu Ser Ser Cys Gly Gly Asp Val Val Pro Phe Ala Gly Lys	
195 200 205	
gga aca aaa gcg ggg tta aag ttt caa acc atg gta aat tgg cta tgt	672
Gly Thr Lys Ala Gly Leu Lys Phe Gln Thr Met Val Asn Trp Leu Cys	
210 215 220	

gaa aac aga gta ttt act gaa gat aaa tgg aaa tta gtg gat ttt aac Glu Asn Arg Val Phe Thr Glu Asp Lys Trp Lys Leu Val Asp Phe Asn 225 230 235 240	720
caa tat act tta tta agt agc agt cac agt ggc agc ttt caa att caa Gln Tyr Thr Leu Leu Ser Ser Ser His Ser Gly Ser Phe Gln Ile Gln 245 250 255	768
agt gcc tta aag tta gct att tat aaa gct act aac tta gta ccc act Ser Ala Leu Lys Leu Ala Ile Tyr Lys Ala Thr Asn Leu Val Pro Thr 260 265 270	816
agt aca ttc ttg tta cat tca gac ttt gag cag gtt act tgc att aaa Ser Thr Phe Leu Leu His Ser Asp Phe Glu Gln Val Thr Cys Ile Lys 275 280 285	864
gaa aat aaa ata gta aaa tta tta ttg tgt caa aac tat gat cct ctt Glu Asn Lys Ile Val Lys Leu Leu Cys Gln Asn Tyr Asp Pro Leu 290 295 300	912
tta gtg ggt caa cat gtg tta agg tgg att gac aaa aaa tgt ggt aaa Leu Val Gly Gln His Val Leu Arg Trp Ile Asp Lys Lys Cys Gly Lys 305 310 315 320	960
aaa aac acc ctg tgg ttt tac ggg cca cca agt act gga aaa aca aat Lys Asn Thr Leu Trp Phe Tyr Gly Pro Ser Thr Gly Lys Thr Asn 325 330 335	1008
ttg gca atg gct att gct aaa act gta cca gtg tat gga atg gtg aat Leu Ala Met Ala Ile Ala Lys Thr Val Pro Val Tyr Gly Met Val Asn 340 345 350	1056
tgg aat aat gaa aac ttt cca ttt aat gat gta gcg ggg aaa agt ttg Trp Asn Asn Glu Asn Phe Pro Phe Asn Asp Val Ala Gly Lys Ser Leu 355 360 365	1104
gtg gtc tgg gat gaa ggc att att aag tcc act att gtg gaa gct gca Val Val Trp Asp Glu Gly Ile Ile Lys Ser Thr Ile Val Glu Ala Ala 370 375 380	1152
aaa gcc att tta ggt ggt cag cca acc agg gta gat cag aaa atg cgt Lys Ala Ile Leu Gly Gly Gln Pro Thr Arg Val Asp Gln Lys Met Arg 385 390 395 400	1200
ggc agt gtg gca gtg ccc ggt gtg cct gtg gtt ata acc agc aat ggt Gly Ser Val Ala Val Pro Gly Val Pro Val Val Ile Thr Ser Asn Gly 405 410 415	1248
gac att aca ttt gtt gtg agt ggt aat acc act aca act gtg cat gct Asp Ile Thr Phe Val Val Ser Gly Asn Thr Thr Thr Thr Val His Ala 420 425 430	1296
aaa gcc tta aag gaa cgg atg gta aag cta aac ttt acc ata aga tgt Lys Ala Leu Lys Glu Arg Met Val Lys Leu Asn Phe Thr Ile Arg Cys 435 440 445	1344
agc cct gac atg ggt tta ctt aca gag gct gat gta caa caa tgg cta Ser Pro Asp Met Gly Leu Thr Glu Ala Asp Val Gln Gln Trp Leu 450 455 460	1392

act tgg tgt aat gca caa agc tgg agc cac tat gaa aac tgg gca ata 1440  
 Thr Trp Cys Asn Ala Gln Ser Trp Ser His Tyr Glu Asn Trp Ala Ile  
 465 470 475 480

aac tac aca ttt gat ttc cct gga ata aat gca gat gcc ctc cac cca 1488  
 Asn Tyr Thr Phe Asp Phe Pro Gly Ile Asn Ala Asp Ala Leu His Pro  
 485 490 495

gat ctc caa acc acc ccc att gtc cca gac acc agt atc agc agc agt 1536  
 Asp Leu Gln Thr Thr Pro Ile Val Pro Asp Thr Ser Ile Ser Ser Ser  
 500 505 510

ggg ggt gaa agc tct gaa gaa ctc agt gaa agc agc ttt ttc aac ctc 1584  
 Gly Gly Glu Ser Ser Glu Glu Ser Glu Ser Ser Phe Phe Asn Leu  
 515 520 525

atc act cca ggc gcc tgg aac agt gaa acc ccg cgc tct agt acg ccc 1632  
 Ile Thr Pro Gly Ala Trp Asn Ser Glu Thr Pro Arg Ser Ser Thr Pro  
 530 535 540

gtc ccc ggg acc agt tca gga gaa tca ttt gtc gga agc cca gtt tcc 1680  
 Val Pro Gly Thr Ser Ser Gly Glu Ser Phe Val Gly Ser Pro Val Ser  
 545 550 555 560

tcc gaa gtg gta gcc gcg tgg gag gaa gct ttt tac acg ccg ctt 1728  
 Ser Glu Val Val Ala Ala Ser Trp Glu Glu Ala Phe Tyr Thr Pro Leu  
 565 570 575

gcc gat cag ttt cgt gaa ctg tta gta ggg gtt gac ttt gta tgg gat 1776  
 Ala Asp Gln Phe Arg Glu Leu Leu Val Gly Val Asp Phe Val Trp Asp  
 580 585 590

ggg ggt agg gga ttg cct gtt tgc tgt gtg gaa cat ata aac aac agt 1824  
 Gly Val Arg Gly Leu Pro Val Cys Cys Val Glu His Ile Asn Asn Ser  
 595 600 605

ggg gga ggg ttg ggg ctt tgc cct cat tgt att aat gtg gga gct tgg 1872  
 Gly Gly Gly Leu Gly Leu Cys Pro His Cys Ile Asn Val Gly Ala Trp  
 610 615 620

tat aat gga tgg aaa ttt aga gag ttt act cca gac tta gtg cgc tgc 1920  
 Tyr Asn Gly Trp Lys Phe Arg Glu Phe Thr Pro Asp Leu Val Arg Cys  
 625 630 635 640

agt tgt cat gta gga gcc tct aac cca ttt tct gtg tta act tgt aaa 1968  
 Ser Cys His Val Gly Ala Ser Asn Pro Phe Ser Val Leu Thr Cys Lys  
 645 650 655

aaa tgt gct tac ctg tct gga tta caa agt ttt gta gat tat gag . 2013  
 Lys Cys Ala Tyr Leu Ser Gly Leu Gln Ser Phe Val Asp Tyr Glu  
 660 665 670

&lt;210&gt; 82

&lt;211&gt; 671

&lt;212&gt; PRT

&lt;213&gt; erythrovirus

&lt;400&gt; 82

Met Glu Leu Phe Arg Gly Val Leu His Ile Ser Ser Asn Ile Leu Asp  
 1 5 10 15  
 Cys Ala Asn Asp Asn Trp Trp Cys Ser Met Leu Asp Leu Asp Thr Ser  
 20 25 30  
 Asp Trp Glu Pro Leu Thr His Ser Asn Arg Leu Met Ala Ile Tyr Leu  
 35 40 45  
 Ser Ser Val Ala Ser Lys Leu Asp Phe Thr Gly Gly Pro Leu Ala Gly  
 50 55 60  
 Cys Leu Tyr Phe Phe Gln Val Glu Cys Asn Lys Phe Glu Glu Gly Tyr  
 65 70 75 80  
 His Ile His Val Val Ile Gly Gly Pro Gly Leu Asn Ala Arg Asn Leu  
 85 90 95  
 Thr Val Cys Val Glu Gly Leu Phe Asn Asn Val Leu Tyr His Leu Val  
 100 105 110  
 Thr Glu Ser Val Lys Leu Lys Phe Leu Pro Gly Met Thr Thr Lys Gly  
 115 120 125  
 Lys Tyr Phe Arg Asp Gly Glu Gln Phe Ile Glu Asn Tyr Leu Met Lys  
 130 135 140  
 Lys Ile Pro Leu Asn Val Val Trp Cys Val Thr Asn Ile Asp Gly Tyr  
 145 150 155 160  
 Ile Asp Thr Cys Ile Ser Ala Ser Phe Arg Arg Gly Ala Cys His Ala  
 165 170 175  
 Lys Arg Pro Arg Ile Thr Ala Asn Thr Asp Ser Ala Thr Asn Glu Thr  
 180 185 190  
 Gly Glu Ser Ser Cys Gly Gly Asp Val Val Pro Phe Ala Gly Lys  
 195 200 205  
 Gly Thr Lys Ala Gly Leu Lys Phe Gln Thr Met Val Asn Trp Leu Cys  
 210 215 220  
 Glu Asn Arg Val Phe Thr Glu Asp Lys Trp Lys Leu Val Asp Phe Asn  
 225 230 235 240  
 Gln Tyr Thr Leu Leu Ser Ser Ser His Ser Gly Ser Phe Gln Ile Gln  
 245 250 255  
 Ser Ala Leu Lys Leu Ala Ile Tyr Lys Ala Thr Asn Leu Val Pro Thr  
 260 265 270  
 Ser Thr Phe Leu Leu His Ser Asp Phe Glu Gln Val Thr Cys Ile Lys  
 275 280 285  
 Glu Asn Lys Ile Val Lys Leu Leu Leu Cys Gln Asn Tyr Asp Pro Leu  
 290 295 300  
 Leu Val Gly Gln His Val Leu Arg Trp Ile Asp Lys Lys Cys Gly Lys  
 305 310 315 320



Lys Asn Thr Leu Trp Phe Tyr Gly Pro Pro Ser Thr Gly Lys Thr Asn  
 325 330 335  
 Leu Ala Met Ala Ile Ala Lys Thr Val Pro Val Tyr Gly Met Val Asn  
 340 345 350  
 Trp Asn Asn Glu Asn Phe Pro Phe Asn Asp Val Ala Gly Lys Ser Leu  
 355 360 365  
 Val Val Trp Asp Glu Gly Ile Ile Lys Ser Thr Ile Val Glu Ala Ala  
 370 375 380  
 Lys Ala Ile Leu Gly Gly Gln Pro Thr Arg Val Asp Gln Lys Met Arg  
 385 390 395 400  
 Gly Ser Val Ala Val Pro Gly Val Pro Val Ile Thr Ser Asn Gly  
 405 410 415  
 Asp Ile Thr Phe Val Val Ser Gly Asn Thr Thr Thr Thr Val His Ala  
 420 425 430  
 Lys Ala Leu Lys Glu Arg Met Val Lys Leu Asn Phe Thr Ile Arg Cys  
 435 440 445  
 Ser Pro Asp Met Gly Leu Leu Thr Glu Ala Asp Val Gln Gln Trp Leu  
 450 455 460  
 Thr Trp Cys Asn Ala Gln Ser Trp Ser His Tyr Glu Asn Trp Ala Ile  
 465 470 475 480  
 Asn Tyr Thr Phe Asp Phe Pro Gly Ile Asn Ala Asp Ala Leu His Pro  
 485 490 495  
 Asp Leu Gln Thr Thr Pro Ile Val Pro Asp Thr Ser Ile Ser Ser Ser  
 500 505 510  
 Gly Gly Glu Ser Ser Glu Glu Leu Ser Glu Ser Ser Phe Phe Asn Leu  
 515 520 525  
 Ile Thr Pro Gly Ala Trp Asn Ser Glu Thr Pro Arg Ser Ser Thr Pro  
 530 535 540  
 Val Pro Gly Thr Ser Ser Gly Glu Ser Phe Val Gly Ser Pro Val Ser  
 545 550 555 560  
 Ser Glu Val Val Ala Ala Ser Trp Glu Glu Ala Phe Tyr Thr Pro Leu  
 565 570 575  
 Ala Asp Gln Phe Arg Glu Leu Leu Val Gly Val Asp Phe Val Trp Asp  
 580 585 590  
 Gly Val Arg Gly Leu Pro Val Cys Cys Val Glu His Ile Asn Asn Ser  
 595 600 605  
 Gly Gly Gly Leu Gly Leu Cys Pro His Cys Ile Asn Val Gly Ala Trp  
 610 615 620

Tyr Asn Gly Trp Lys Phe Arg Glu Phe Thr Pro Asp Leu Val Arg Cys  
625 630 635 640

Ser Cys His Val Gly Ala Ser Asn Pro Phe Ser Val Leu Thr Cys Lys  
645 650 655

Lys Cys Ala Tyr Leu Ser Gly Leu Gln Ser Phe Val Asp Tyr Glu  
660 665 670

<210> 83

<211> 222

<212> DNA

<213> erythrovirus

<400> 83

atg cag atg ccc tcc acc cag atc tcc aaa cca ccc cca ttg tcc cag 48  
Met Gln Met Pro Ser Thr Gln Ile Ser Lys Pro Pro Pro Leu Ser Gln  
675 680 685

aca cca gta tca gca gca gtg gtg gtg aaa gct ctg aag aac tca gtg 96  
Thr Pro Val Ser Ala Ala Val Val Lys Ala Leu Lys Asn Ser Val  
690 695 700

aaa gca gct ttt tca acc tca tca ctc cag gcg cct gga aca gtg aaa 144  
Lys Ala Ala Phe Ser Thr Ser Ser Leu Gln Ala Pro Gly Thr Val Lys  
705 710 715

ccc cgc gct cta gta cgc ccg tcc ccg gga cca gtt cag gag aat cat 192  
Pro Arg Ala Leu Val Arg Pro Ser Pro Gly Pro Val Gln Glu Asn His  
720 725 730 735

ttg tcg gaa gcc cag ttt cct ccg aag tgg 222  
Leu Ser Glu Ala Gln Phe Pro Pro Lys Trp  
740 745

<210> 84

<211> 74

<212> PRT

<213> erythrovirus

<400> 84

Met Gln Met Pro Ser Thr Gln Ile Ser Lys Pro Pro Pro Leu Ser Gln  
1 5 10 15

Thr Pro Val Ser Ala Ala Val Val Val Lys Ala Leu Lys Asn Ser Val  
20 25 30

Lys Ala Ala Phe Ser Thr Ser Ser Leu Gln Ala Pro Gly Thr Val Lys  
35 40 45

Pro Arg Ala Leu Val Arg Pro Ser Pro Gly Pro Val Gln Glu Asn His  
50 55 60

Leu Ser Glu Ala Gln Phe Pro Pro Lys Trp  
65 70

<210> 85

<211> 2343

<212> DNA

## &lt;213&gt; erythrovirus

&lt;400&gt; 85

atg agt aaa acc act aac aaa tgg tgg gaa agc agt gac aaa ttt gcc 48  
 Met Ser Lys Thr Thr Asn Lys Trp Trp Glu Ser Ser Asp Lys Phe Ala  
 75 80 85 90

cag gac gtg tat aag cag ttt gtg caa ttt tat gaa aaa gct act gga 96  
 Gln Asp Val Tyr Lys Gln Phe Val Gln Phe Tyr Glu Lys Ala Thr Gly  
 95 100 105

aca gac tta gag ctt att caa att tta aaa gac cat tac aac att tct 144  
 Thr Asp Leu Glu Leu Ile Gln Ile Leu Lys Asp His Tyr Asn Ile Ser  
 110 115 120

tta gat aat cct tta gaa aac ccc tct tct tta ttt gac tta gtt gct 192  
 Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser Leu Phe Asp Leu Val Ala  
 125 130 135

cgc att aaa agt aat ctt aaa aac tct cca gac cta tat agt cat cat 240  
 Arg Ile Lys Ser Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His His  
 140 145 150

ttt cag agc cat gga cag tta tct gac cac ccc cat gcc tta tca tcc 288  
 Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala Leu Ser Ser  
 155 160 165 170

agt aac agt agt gca gaa cct aga gga gaa aat gca gta tta tct agt 336  
 Ser Asn Ser Ser Ala Glu Pro Arg Gly Glu Asn Ala Val Leu Ser Ser  
 175 180 185

gaa gac tta cac aag cct ggg caa gtt agc ata caa tta ccc ggt act 384  
 Glu Asp Leu His Lys Pro Gly Gln Val Ser Ile Gln Leu Pro Gly Thr  
 190 195 200

aac tat gtt ggg cct ggc aat gag cta caa gct ggg cct ccg cag aat 432  
 Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Gln Asn  
 205 210 215

gct gtg gac agt gct gca agg att cat gac ttt agg tat agc caa ttg 480  
 Ala Val Asp Ser Ala Ala Arg Ile His Asp Phe Arg Tyr Ser Gln Leu  
 220 225 230

gct aag ttg gga ata aat cct tat aca cat tgg acg gta gca gat gaa 528  
 Ala Lys Leu Gly Ile Asn Pro Tyr Thr His Trp Thr Val Ala Asp Glu  
 235 240 245 250

gaa ttg tta aaa aat ata aaa aat gaa aca ggg ttt caa gca caa gca 576  
 Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Gly Phe Gln Ala Gln Ala  
 255 260 265

gta aaa gat tac ttt act tta aaa ggt gca gct gcc cct gtg gcc cat 624  
 Val Lys Asp Tyr Phe Thr Leu Lys Gly Ala Ala Ala Pro Val Ala His  
 270 275 280

ttt caa gga agt tta ccg gaa gtg ccc gcg tac aac gcc tca gaa aaa 672  
 Phe Gln Gly Ser Leu Pro Glu Val Pro Ala Tyr Asn Ala Ser Glu Lys  
 285 290 295

tac ccc agc atg act tca gtt aac tct gca gaa gcc agc act ggt gca Tyr Pro Ser Met Thr Ser Val Asn Ser Ala Glu Ala Ser Thr Gly Ala 300 305 310	720
ggc ggg gga ggt agc aac cct aca aaa agc atg tgg agt gaa ggg gct Gly Gly Gly Gly Ser Asn Pro Thr Lys Ser Met Trp Ser Glu Gly Ala 315 320 325 330	768
aca ttt act gct aat tct gta acg tgt aca ttc tct agg caa ttt tta Thr Phe Thr Ala Asn Ser Val Thr Cys Thr Phe Ser Arg Gln Phe Leu 335 340 345	816
att cca tat gat cca gag cat cat tat aaa gtg ttc tct cca gca gct Ile Pro Tyr Asp Pro Glu His His Tyr Lys Val Phe Ser Pro Ala Ala 350 355 360	864
agt agc tgc cac aat gct agt ggg aaa gag gca aaa gtg tgc act att Ser Ser Cys His Asn Ala Ser Gly Lys Glu Ala Lys Val Cys Thr Ile 365 370 375	912
agt ccc att atg ggg tac tct act ccg tgg aga tac tta gat ttt aat Ser Pro Ile Met Gly Tyr Ser Thr Pro Trp Arg Tyr Leu Asp Phe Asn 380 385 390	960
gct tta aat ttg ttt ttc tca cca tta gag ttt cag cac tta att gaa Ala Leu Asn Leu Phe Phe Ser Pro Leu Glu Phe Gln His Leu Ile Glu 395 400 405 410	1008
aat tat ggt agt ata gct cca gat gct tta act gta act att tca gaa Asn Tyr Gly Ser Ile Ala Pro Asp Ala Leu Thr Val Thr Ile Ser Glu 415 420 425	1056
att gct gta aaa gat gtc aca gac aaa aca gga gga ggt gtg caa gtt Ile Ala Val Lys Asp Val Thr Asp Lys Thr Gly Gly Gly Val Gln Val 430 435 440	1104
act gac agc acc aca gga cgt ttg tgt atg tta gtg gat cat gag tat Thr Asp Ser Thr Thr Gly Arg Leu Cys Met Leu Val Asp His Glu Tyr 445 450 455	1152
aaa tac cca tat gtg cta ggt cag gga caa gac aca cta gct cca gaa Lys Tyr Pro Tyr Val Leu Gly Gln Gly Gln Asp Thr Leu Ala Pro Glu 460 465 470	1200
ctg ccc att tgg gtt tac ttt ccc ccc cag tat gct tac tta aca gta Leu Pro Ile Trp Val Tyr Phe Pro Pro Gln Tyr Ala Tyr Leu Thr Val 475 480 485 490	1248
ggc gaa gta aac aca caa gga att tca gga gac agc aaa aaa ttg gct Gly Glu Val Asn Thr Gln Gly Ile Ser Gly Asp Ser Lys Lys Leu Ala 495 500 505	1296
agt gaa gaa tca gct ttt tat gtg tta gag cac agt tca ttt gaa ctt Ser Glu Glu Ser Ala Phe Tyr Val Leu Glu His Ser Ser Phe Glu Leu 510 515 520	1344
ttg ggt aca ggg gga tct gcc act atg tcc tac aaa ttt cca gct gtg Leu Gly Thr Gly Gly Ser Ala Thr Met Ser Tyr Lys Phe Pro Ala Val 525 530 535	1392

ccc cca gaa aac cta gaa ggc tgc agc caa cat ttt tat gaa atg tac Pro Pro Glu Asn Leu Glu Gly Cys Ser Gln His Phe Tyr Glu Met Tyr 540 545 550	1440
aac cct ttg tac ggt tct cgt tta ggg gta cct gac aca tta gga ggg Asn Pro Leu Tyr Gly Ser Arg Leu Gly Val Pro Asp Thr Leu Gly Gly 555 560 565 570	1488
gac cct aaa ttt aga tca ttg aca cac gaa gac cac gca att cag cca Asp Pro Lys Phe Arg Ser Leu Thr His Glu Asp His Ala Ile Gln Pro 575 580 585	1536
caa aac ttt atg cct ggg cca cta ata aat tca gtg tct acc aaa gaa Gln Asn Phe Met Pro Gly Pro Leu Ile Asn Ser Val Ser Thr Lys Glu 590 595 600	1584
gga gac aat tct aat aca ggt gct gga aaa gcc ctt acg ggg ctt agt Gly Asp Asn Ser Asn Thr Gly Ala Gly Lys Ala Leu Thr Gly Leu Ser 605 610 615	1632
act ggc act agc caa aac acc aga att tcc cta cgc ccc ggg cca gta Thr Gly Thr Ser Gln Asn Thr Arg Ile Ser Leu Arg Pro Gly Pro Val 620 625 630	1680
tct cag cca tac cat cac tgg gac act gat aaa tat gtt aca gga ata Ser Gln Pro Tyr His His Trp Asp Thr Asp Lys Tyr Val Thr Gly Ile 635 640 645 650	1728
aat gcc att tca cat gga caa acc act tat gga aat gct gag gac aaa Asn Ala Ile Ser His Gly Gln Thr Thr Tyr Gly Asn Ala Glu Asp Lys 655 660 665	1776
gag tat cag caa ggg gta gga aga ttt cca aat gaa aaa gaa cag ctt Glu Tyr Gln Gln Gly Val Gly Arg Phe Pro Asn Glu Lys Glu Gln Leu 670 675 680	1824
aag cag tta caa ggt ctt aac atg cac aca tac ttc cct aat aaa gga Lys Gln Leu Gln Gly Leu Asn Met His Thr Tyr Phe Pro Asn Lys Gly 685 690 695	1872
acc caa caa tac aca gac caa att gaa cgc cct ctt atg gtg ggc tct Thr Gln Gln Tyr Thr Asp Gln Ile Glu Arg Pro Leu Met Val Gly Ser 700 705 710	1920
gtt tgg aac aga aga gct ctt cac tat gaa agt cag ctg tgg agt aaa Val Trp Asn Arg Arg Ala Leu His Tyr Glu Ser Gln Leu Trp Ser Lys 715 720 725 730	1968
atc cct aac tta gat gac agt ttt aaa act caa ttt gca gcc cta ggc Ile Pro Asn Leu Asp Asp Ser Phe Lys Thr Gln Phe Ala Ala Leu Gly 735 740 745	2016
ggg tgg ggt ttg cat caa cca ccc cct caa ata ttt tta aaa ata cta Gly Trp Gly Leu His Gln Pro Pro Pro Gln Ile Phe Leu Lys Ile Leu 750 755 760	2064

cca	caa	agt	ggg	cca	att	gga	ggg	att	aaa	tcc	atg	gga	att	act	act		2112
Pro	Gln	Ser	Gly	Pro	Ile	Gly	Gly	Ile	Lys	Ser	Met	Gly	Ile	Thr	Thr		
		765					770					775					
tta	gtt	caa	tat	gct	gtg	gga	ata	atg	aca	gtt	acc	atg	acc	ttt	aaa		2160
Leu	Val	Gln	Tyr	Ala	Val	Gly	Ile	Met	Thr	Val	Thr	Met	Thr	Phe	Lys		
		780				785					790						
ttg	gga	cct	cga	aag	gct	act	gga	agg	tgg	aat	ccc	cag	cct	ggc	gtt		2208
Leu	Gly	Pro	Arg	Lys	Ala	Thr	Gly	Arg	Trp	Asn	Pro	Gln	Pro	Gly	Val		
		795			800					805					810		
tat	cct	cct	cat	gca	gct	ggg	cat	tta	cca	tat	gta	ctg	tat	gac	ccc		2256
Tyr	Pro	Pro	His	Ala	Ala	Gly	His	Leu	Pro	Tyr	Val	Leu	Tyr	Asp	Pro		
				815					820					825			
aca	gct	aca	gat	gca	aag	caa	cac	cac	aga	cac	gga	tat	gaa	aag	cct		2304
Thr	Ala	Thr	Asp	Ala	Lys	Gln	His	His	Arg	His	Gly	Tyr	Glu	Lys	Pro		
			830					835					840				
gaa	gaa	ttg	tgg	act	gcc	aaa	agc	cgt	gtg	cac	cca	ttg					2343
Glu	Glu	Leu	Trp	Thr	Ala	Lys	Ser	Arg	Val	His	Pro	Leu					
		845					850					855					
<210> 86																	
<211> 781																	
<212> PRT																	
<213> erythrovirus																	
<400> 86																	
Met	Ser	Lys	Thr	Thr	Asn	Lys	Trp	Trp	Glu	Ser	Ser	Asp	Lys	Phe	Ala		
	1			5					10					15			
Gln	Asp	Val	Tyr	Lys	Gln	Phe	Val	Gln	Phe	Tyr	Glu	Lys	Ala	Thr	Gly		
		20						25					30				
Thr	Asp	Leu	Glu	Leu	Ile	Gln	Ile	Leu	Lys	Asp	His	Tyr	Asn	Ile	Ser		
		35					40					45					
Leu	Asp	Asn	Pro	Leu	Glu	Asn	Pro	Ser	Ser	Leu	Phe	Asp	Leu	Val	Ala		
		50				55					60						
Arg	Ile	Lys	Ser	Asn	Leu	Lys	Asn	Ser	Pro	Asp	Leu	Tyr	Ser	His	His		
	65				70					75					80		
Phe	Gln	Ser	His	Gly	Gln	Leu	Ser	Asp	His	Pro	His	Ala	Leu	Ser	Ser		
				85					90					95			
Ser	Asn	Ser	Ser	Ala	Glu	Pro	Arg	Gly	Glu	Asn	Ala	Val	Leu	Ser	Ser		
			100					105					110				
Glu	Asp	Leu	His	Lys	Pro	Gly	Gln	Val	Ser	Ile	Gln	Leu	Pro	Gly	Thr		
		115					120					125					
Asn	Tyr	Val	Gly	Pro	Gly	Asn	Glu	Leu	Gln	Ala	Gly	Pro	Pro	Gln	Asn		

Ala Lys Leu Gly Ile Asn Pro Tyr Thr His Trp Thr Val Ala Asp Glu  
165 170 175

Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Gly Phe Gln Ala Gln Ala  
180 185 190

Val Lys Asp Tyr Phe Thr Leu Lys Gly Ala Ala Ala Pro Val Ala His  
195 200 205

Phe Gln Gly Ser Leu Pro Glu Val Pro Ala Tyr Asn Ala Ser Glu Lys  
210 215 220

Tyr Pro Ser Met Thr Ser Val Asn Ser Ala Glu Ala Ser Thr Gly Ala  
225 230 235 240

Gly Gly Gly Gly Ser Asn Pro Thr Lys Ser Met Trp Ser Glu Gly Ala  
245 250 255

Thr Phe Thr Ala Asn Ser Val Thr Cys Thr Phe Ser Arg Gln Phe Leu  
260 265 270

Ile Pro Tyr Asp Pro Glu His His Tyr Lys Val Phe Ser Pro Ala Ala  
275 280 285

Ser Ser Cys His Asn Ala Ser Gly Lys Glu Ala Lys Val Cys Thr Ile  
290 295 300

Ser Pro Ile Met Gly Tyr Ser Thr Pro Trp Arg Tyr Leu Asp Phe Asn  
305 310 315 320

Ala Leu Asn Leu Phe Phe Ser Pro Leu Glu Phe Gln His Leu Ile Glu  
325 330 335

Asn Tyr Gly Ser Ile Ala Pro Asp Ala Leu Thr Val Thr Ile Ser Glu  
340 345 350

Ile Ala Val Lys Asp Val Thr Asp Lys Thr Gly Gly Gly Val Gln Val  
355 360 365

Thr Asp Ser Thr Thr Gly Arg Leu Cys Met Leu Val Asp His Glu Tyr  
370 375 380

Lys Tyr Pro Tyr Val Leu Gly Gln Gly Gln Asp Thr Leu Ala Pro Glu  
385 390 395 400

Leu Pro Ile Trp Val Tyr Phe Pro Pro Gln Tyr Ala Tyr Leu Thr Val  
405 410 415

Gly Glu Val Asn Thr Gln Gly Ile Ser Gly Asp Ser Lys Lys Leu Ala  
420 425 430

Ser Glu Glu Ser Ala Phe Tyr Val Leu Glu His Ser Ser Phe Glu Leu  
435 440 445

Leu Gly Thr Gly Gly Ser Ala Thr Met Ser Tyr Lys Phe Pro Ala Val  
450 455 460

Pro Pro Glu Asn Leu Glu Gly Cys Ser Gln His Phe Tyr Glu Met Tyr  
 465 470 475 480  
 Asn Pro Leu Tyr Gly Ser Arg Leu Gly Val Pro Asp Thr Leu Gly Gly  
 485 490 495  
 Asp Pro Lys Phe Arg Ser Leu Thr His Glu Asp His Ala Ile Gln Pro  
 500 505 510  
 Gln Asn Phe Met Pro Gly Pro Leu Ile Asn Ser Val Ser Thr Lys Glu  
 515 520 525  
 Gly Asp Asn Ser Asn Thr Gly Ala Gly Lys Ala Leu Thr Gly Leu Ser  
 530 535 540  
 Thr Gly Thr Ser Gln Asn Thr Arg Ile Ser Leu Arg Pro Gly Pro Val  
 545 550 555 560  
 Ser Gln Pro Tyr His His Trp Asp Thr Asp Lys Tyr Val Thr Gly Ile  
 565 570 575  
 Asn Ala Ile Ser His Gly Gln Thr Thr Tyr Gly Asn Ala Glu Asp Lys  
 580 585 590  
 Glu Tyr Gln Gln Gly Val Gly Arg Phe Pro Asn Glu Lys Glu Gln Leu  
 595 600 605  
 Lys Gln Leu Gln Gly Leu Asn Met His Thr Tyr Phe Pro Asn Lys Gly  
 610 615 620  
 Thr Gln Gln Tyr Thr Asp Gln Ile Glu Arg Pro Leu Met Val Gly Ser  
 625 630 635 640  
 Val Trp Asn Arg Arg Ala Leu His Tyr Glu Ser Gln Leu Trp Ser Lys  
 645 650 655  
 Ile Pro Asn Leu Asp Asp Ser Phe Lys Thr Gln Phe Ala Ala Leu Gly  
 660 665 670  
 Gly Trp Gly Leu His Gln Pro Pro Pro Gln Ile Phe Leu Lys Ile Leu  
 675 680 685  
 Pro Gln Ser Gly Pro Ile Gly Gly Ile Lys Ser Met Gly Ile Thr Thr  
 690 695 700  
 Leu Val Gln Tyr Ala Val Gly Ile Met Thr Val Thr Met Thr Phe Lys  
 705 710 715 720  
 Leu Gly Pro Arg Lys Ala Thr Gly Arg Trp Asn Pro Gln Pro Gly Val  
 725 730 735  
 Tyr Pro Pro His Ala Ala Gly His Leu Pro Tyr Val Leu Tyr Asp Pro  
 740 745 750  
 Thr Ala Thr Asp Ala Lys Gln His His Arg His Gly Tyr Glu Lys Pro  
 755 760 765  
 Glu Glu Leu Trp Thr Ala Lys Ser Arg Val His Pro Leu  
 770 775 780



<210> 87  
 <211> 681  
 <212> DNA  
 <213> erythrovirus

<400> 87  
 atg agt aaa acc act aac aaa tgg tgg gaa agc agt gac aaa ttt gcc 48  
 Met Ser Lys Thr Thr Asn Lys Trp Trp Glu Ser Ser Asp Lys Phe Ala  
                     785                    790                    795

cag gac gtg tat aag cag ttt gtg caa ttt tat gaa aaa gct act gga 96  
 Gln Asp Val Tyr Lys Gln Phe Val Gln Phe Tyr Glu Lys Ala Thr Gly  
                     800                    805                    810

aca gac tta gag ctt att caa att tta aaa gac cat tac aac att tct 144  
 Thr Asp Leu Glu Leu Ile Gln Ile Leu Lys Asp His Tyr Asn Ile Ser  
                     815                    820                    825

tta gat aat cct tta gaa aac ccc tct tct tta ttt gac tta gtt gct 192  
 Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser Leu Phe Asp Leu Val Ala  
                     830                    835                    840                    845

cgc att aaa agt aat ctt aaa aac tct cca gac cta tat agt cat cat 240  
 Arg Ile Lys Ser Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His His  
                     850                    855                    860

ttt cag agc cat gga cag tta tct gac cac ccc cat gcc tta tca tcc 288  
 Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala Leu Ser Ser  
                     865                    870                    875

agt aac agt agt gca gaa cct aga gga gaa aat gca gta tta tct agt 336  
 Ser Asn Ser Ser Ala Glu Pro Arg Gly Glu Asn Ala Val Leu Ser Ser  
                     880                    885                    890

gaa gac tta cac aag cct ggg caa gtt agc ata caa tta ccc ggt act 384  
 Glu Asp Leu His Lys Pro Gly Gln Val Ser Ile Gln Leu Pro Gly Thr  
                     895                    900                    905

aac tat gtt ggg cct ggc aat gag cta caa gct ggg cct cag aat 432  
 Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln Asn  
                     910                    915                    920                    925

gct gtg gac agt gct gca agg att cat gac ttt agg tat agc caa ttg 480  
 Ala Val Asp Ser Ala Ala Arg Ile His Asp Phe Arg Tyr Ser Gln Leu  
                     930                    935                    940

gct aag ttg gga ata aat cct tat aca cat tgg acg gta gca gat gaa 528  
 Ala Lys Leu Gly Ile Asn Pro Tyr Thr His Trp Thr Val Ala Asp Glu  
                     945                    950                    955

gaa ttg tta aaa aat ata aaa aat gaa aca ggg ttt caa gca caa gca 576  
 Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Gly Phe Gln Ala Gln Ala  
                     960                    965                    970

gta aaa gat tac ttt act tta aaa ggt gca gct gcc cct gtg gcc cat 624  
 Val Lys Asp Tyr Phe Thr Leu Lys Gly Ala Ala Ala Pro Val Ala His  
                     975                    980                    985

ttt caa gga agt tta ccg gaa gtg ccc gcg tac aac gcc tca gaa aaa 672  
 Phe Gln Gly Ser Leu Pro Glu Val Pro Ala Tyr Asn Ala Ser Glu Lys  
 990 995 1000 1005

tac ccc agc 681  
 Tyr Pro Ser

<210> 88  
 <211> 227  
 <212> PRT  
 <213> erythrovirus

<400> 88  
 Met Ser Lys Thr Thr Asn Lys Trp Trp Glu Ser Ser Asp Lys Phe Ala  
 1 5 10 15  
 Gln Asp Val Tyr Lys Gln Phe Val Gln Phe Tyr Glu Lys Ala Thr Gly  
 20 25 30  
 Thr Asp Leu Glu Leu Ile Gln Ile Leu Lys Asp His Tyr Asn Ile Ser  
 35 40 45  
 Leu Asp Asn Pro Leu Glu Asn Pro Ser Ser Leu Phe Asp Leu Val Ala  
 50 55 60  
 Arg Ile Lys Ser Asn Leu Lys Asn Ser Pro Asp Leu Tyr Ser His His  
 65 70 75 80  
 Phe Gln Ser His Gly Gln Leu Ser Asp His Pro His Ala Leu Ser Ser  
 85 90 95  
 Ser Asn Ser Ser Ala Glu Pro Arg Gly Glu Asn Ala Val Leu Ser Ser  
 100 105 110  
 Glu Asp Leu His Lys Pro Gly Gln Val Ser Ile Gln Leu Pro Gly Thr  
 115 120 125  
 Asn Tyr Val Gly Pro Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln Asn  
 130 135 140  
 Ala Val Asp Ser Ala Ala Arg Ile His Asp Phe Arg Tyr Ser Gln Leu  
 145 150 155 160  
 Ala Lys Leu Gly Ile Asn Pro Tyr Thr His Trp Thr Val Ala Asp Glu  
 165 170 175  
 Glu Leu Leu Lys Asn Ile Lys Asn Glu Thr Gly Phe Gln Ala Gln Ala  
 180 185 190  
 Val Lys Asp Tyr Phe Thr Leu Lys Gly Ala Ala Ala Pro Val Ala His  
 195 200 205  
 Phe Gln Gly Ser Leu Pro Glu Val Pro Ala Tyr Asn Ala Ser Glu Lys  
 210 215 220  
 Tyr Pro Ser  
 225

<210> 89  
 <211> 306  
 <212> DNA  
 <213> erythrovirus

<400> 89  
 ttg ctc gca tta aaa gta atc tta aaa act ctc cag acc tat ata gtc 48  
 Leu Leu Ala Leu Lys Val Ile Leu Lys Thr Leu Gln Thr Tyr Ile Val  
           230                  235                  240

atc att ttc aga gcc atg gac agt tat ctg acc acc ccc atg cct tat 96  
 Ile Ile Phe Arg Ala Met Asp Ser Tyr Leu Thr Thr Pro Met Pro Tyr  
           245                  250                  255

cat cca gta aca gta gtg cag aac cta gag gag aaa atg cag tat tat 144  
 His Pro Val Thr Val Val Gln Asn Leu Glu Lys Met Gln Tyr Tyr  
           260                  265                  270                  275

cta gtg aag act tac aca agc ctg ggc aag tta gca tac aat tac ccg 192  
 Leu Val Lys Thr Tyr Thr Ser Leu Gly Lys Leu Ala Tyr Asn Tyr Pro  
                           280                  285                          290

gta cta act atg ttg ggc ctg gca atg agc tac aag ctg ggc ctc cgc 240  
 Val Leu Thr Met Leu Gly Leu Ala Met Ser Tyr Lys Leu Gly Leu Arg  
                           295                  300                          305

aga atg ctg tgg aca gtg ctg caa gga ttc atg act tta ggt ata gcc 288  
 Arg Met Leu Trp Thr Val Leu Gln Gly Phe Met Thr Leu Gly Ile Ala  
                           310                  315                          320

aat tgg cta agt tgg gaa 306  
 Asn Trp Leu Ser Trp Glu  
           325

<210> 90  
 <211> 102  
 <212> PRT  
 <213> erythrovirus

<400> 90  
 Leu Leu Ala Leu Lys Val Ile Leu Lys Thr Leu Gln Thr Tyr Ile Val  
   1                  5                  10                  15

Ile Ile Phe Arg Ala Met Asp Ser Tyr Leu Thr Thr Pro Met Pro Tyr  
                   20                  25                  30

His Pro Val Thr Val Val Gln Asn Leu Glu Glu Lys Met Gln Tyr Tyr  
           35                  40                  45

Leu Val Lys Thr Tyr Thr Ser Leu Gly Lys Leu Ala Tyr Asn Tyr Pro  
   50                  55                  60

Val Leu Thr Met Leu Gly Leu Ala Met Ser Tyr Lys Leu Gly Leu Arg  
   65                  70                  75                  80

Arg Met Leu Trp Thr Val Leu Gln Gly Phe Met Thr Leu Gly Ile Ala  
                   85                  90                  95

Asn Trp Leu Ser Trp Glu  
100

<210> 91  
<211> 1662  
<212> DNA  
<213> erythrovirus

<400> 91  
atg act tca gtt aac tct gca gaa gcc agc act ggt gca ggc ggg gga 48  
Met Thr Ser Val Asn Ser Ala Glu Ala Ser Thr Gly Ala Gly Gly Gly  
105 110 115

ggc agc aac cct aca aaa agc atg tgg agt gaa ggg gct aca ttt act 96  
Gly Ser Asn Pro Thr Lys Ser Met Trp Ser Glu Gly Ala Thr Phe Thr  
120 125 130

gct aat tct gta acg tgt aca ttc tct agg caa ttt tta att cca tat 144  
Ala Asn Ser Val Thr Cys Thr Phe Ser Arg Gln Phe Leu Ile Pro Tyr  
135 140 145 150

gat cca gag cat cat tat aaa gtg ttc tct cca gca gct agt agc tgc 192  
Asp Pro Glu His His Tyr Lys Val Phe Ser Pro Ala Ala Ser Ser Cys  
155 160 165

cac aat gct agt ggg aaa gag gca aaa gtg tgc act att agt ccc att 240  
His Asn Ala Ser Gly Lys Glu Ala Lys Val Cys Thr Ile Ser Pro Ile  
170 175 180

atg ggg tac tct act cgg tgg aga tac tta gat ttt aat gct tta aat 288  
Met Gly Tyr Ser Thr Pro Trp Arg Tyr Leu Asp Phe Asn Ala Leu Asn  
185 190 195

ttg ttt ttc tca cca tta gag ttt cag cac tta att gaa aat tat ggt 336  
Leu Phe Phe Ser Pro Leu Glu Phe Gln His Leu Ile Glu Asn Tyr Gly  
200 205 210

agt ata gct cca gat gct tta act gta act att tca gaa att gct gta 384  
Ser Ile Ala Pro Asp Ala Leu Thr Val Thr Ile Ser Glu Ile Ala Val  
215 220 225 230

aaa gat gtc aca gac aaa aca gga gga ggt gtg caa gtt act gac agc 432  
Lys Asp Val Thr Asp Lys Thr Gly Gly Gly Val Gln Val Thr Asp Ser  
235 240 245

acc aca gga cgt ttg tgt atg tta gtg gat cat gag tat aaa tac cca 480  
Thr Thr Gly Arg Leu Cys Met Leu Val Asp His Glu Tyr Lys Tyr Pro  
250 255 260

tat gtg cta ggt cag gga caa gac aca cta gct cca gaa ctg cco att 528  
Tyr Val Leu Gly Gln Gly Thr Leu Ala Pro Glu Leu Pro Ile  
265 270 275

tgg gtt tac ttt ccc ccc cag tat gct tac tta aca gta ggt gaa gta 576  
Trp Val Tyr Phe Pro Pro Gln Tyr Thr Ala Tyr Leu Thr Val Gly Glu Val  
280 285 290

aac aca caa gga att tca gga gac agc aaa aaa ttg gct agt gaa gaa Asn Thr Gln Gly Ile Ser Gly Asp Ser Lys Leu Ala Ser Glu Glu 295 300 305 310	624
tca gct ttt tat gtg tta gag cac agt tca ttt gaa ctt ttg ggt aca Ser Ala Phe Tyr Val Leu Glu His Ser Ser Phe Glu Leu Leu Gly Thr 315 320 325	672
ggg gga tct gcc act atg tcc tac aaa ttt cca gct gtg ccc cca gaa Gly Gly Ser Ala Thr Met Ser Tyr Lys Phe Pro Ala Val Pro Pro Glu 330 335 340	720
aac cta gaa ggc tgc agc caa cat ttt tat gaa atg tac aac cct ttg Asn Leu Glu Gly Cys Ser Gln His Phe Tyr Glu Met Tyr Asn Pro Leu 345 350 355	768
tac ggt tct cgt tta ggg gta cct gac aca tta gga ggg gac cct aaa Tyr Gly Ser Arg Leu Gly Val Pro Asp Thr Leu Gly Gly Asp Pro Lys 360 365 370	816
ttt aga tca ttg aca cac gaa gac cac gca att cag cca caa aac ttt Phe Arg Ser Leu Thr His Glu Asp His Ala Ile Gln Pro Gln Asn Phe 375 380 385 390	864
atg cct ggg cca cta ata aat tca gtg tct acc aaa gaa gga gac aat Met Pro Gly Pro Leu Ile Asn Ser Val Ser Thr Lys Glu Gly Asp Asn 395 400 405	912
tct aat aca ggt gct gga aaa gcc ctt acg ggg ctt agt act ggc act Ser Asn Thr Gly Ala Gly Lys Ala Leu Thr Gly Leu Ser Thr Gly Thr 410 415 420	960
agc caa aac acc aga att tcc cta cgc ccc ggg cca gta tct cag cca Ser Gln Asn Thr Arg Ile Ser Leu Arg Pro Gly Pro Val Ser Gln Pro 425 430 435	1008
tac cat cac tgg gac act gat aaa tat gtt aca gga ata aat gcc att Tyr His His Trp Asp Thr Asp Lys Tyr Val Thr Gly Ile Asn Ala Ile 440 445 450	1056
tca cat gga caa acc act tat gga aat gct gag gac aaa gag tat cag Ser His Gly Gln Thr Thr Tyr Gly Asn Ala Glu Asp Lys Glu Tyr Gln 455 460 465 470	1104
caa ggg gta gga aga ttt cca aat gaa aaa gaa cag ctt aag cag tta Gln Gly Val Gly Arg Phe Pro Asn Glu Lys Glu Gln Leu Lys Gln Leu 475 480 485	1152
caa ggt ctt aac atg cac aca tac ttc cct aat aaa gga acc caa caa Gln Gly Leu Asn Met His Thr Tyr Phe Pro Asn Lys Gly Thr Gln Gln 490 495 500	1200
tac aca gac caa att gaa cgc cct ctt atg gtg ggc tct gtt tgg aac Tyr Thr Asp Gln Ile Glu Arg Pro Leu Met Val Gly Ser Val Trp Asn 505 510 515	1248
aga aga gct ctt cac tat gaa agt cag ctg tgg agt aaa atc cct aac Arg Arg Ala Leu His Tyr Glu Ser Gln Leu Trp Ser Lys Ile Pro Asn 520 525 530	1296

tta gat gac agt ttt aaa act caa ttt gca gcc cta ggc ggg tgg ggt 1344  
 Leu Asp Asp Ser Phe Lys Thr Gln Phe Ala Ala Leu Gly Gly Trp Gly  
 535 540 545 550

ttg cat caa cca ccc cct caa ata ttt tta aaa ata cta cca caa agt 1392  
 Leu His Gln Pro Pro Pro Gln Ile Phe Leu Lys Ile Leu Pro Gln Ser  
 555 560 565

ggg cca att gga ggt att aaa tcc atg gga att act act tta gtt caa 1440  
 Gly Pro Ile Gly Gly Ile Lys Ser Met Gly Ile Thr Thr Leu Val Gln  
 570 575 580

tat gct gtg gga ata atg aca gtt acc atg acc ttt aaa ttg gga cct 1488  
 Tyr Ala Val Gly Ile Met Thr Val Thr Met Thr Phe Lys Leu Gly Pro  
 585 590 595

cga aag gct act gga agg tgg aat ccc cag cct ggc gtt tat cct cct 1536  
 Arg Lys Ala Thr Gly Arg Trp Asn Pro Gln Pro Gly Val Tyr Pro Pro  
 600 605 610

cat gca gct ggt cat tta cca tat gta ctg tat gac ccc aca gct aca 1584  
 His Ala Ala Gly His Leu Pro Tyr Val Leu Tyr Asp Pro Thr Ala Thr  
 615 620 625 630

gat gca aag caa cac cac aga cac gga tat gaa aag cct gaa gaa ttg 1632  
 Asp Ala Lys Gln His His Arg His Gly Tyr Glu Lys Pro Glu Glu Leu  
 635 640 645

tgg act gcc aaa agc cgt gtg cac cca ttg 1662  
 Trp Thr Ala Lys Ser Arg Val His Pro Leu  
 650 655

<210> 92  
 <211> 554  
 <212> PRT  
 <213> erythrovirus

<400> 92  
 Met Thr Ser Val Asn Ser Ala Glu Ala Ser Thr Gly Ala Gly Gly Gly  
 1 5 10 15

Gly Ser Asn Pro Thr Lys Ser Met Trp Ser Glu Gly Ala Thr Phe Thr  
 20 25 30

Ala Asn Ser Val Thr Cys Thr Phe Ser Arg Gln Phe Leu Ile Pro Tyr  
 35 40 45

Asp Pro Glu His His Tyr Lys Val Phe Ser Pro Ala Ala Ser Ser Cys  
 50 55 60

His Asn Ala Ser Gly Lys Glu Ala Lys Val Cys Thr Ile Ser Pro Ile  
 65 70 75 80

Met Gly Tyr Ser Thr Pro Trp Arg Tyr Leu Asp Phe Asn Ala Leu Asn  
 85 90 95

Leu Phe Phe Ser Pro Leu Glu Phe Gln His Leu Ile Glu Asn Tyr Gly  
 100 105 110

Ser Ile Ala Pro Asp Ala Leu Thr Val Thr Ile Ser Glu Ile Ala Val  
 115 120 125  
 Lys Asp Val Thr Asp Lys Thr Gly Gly Gly Val Gln Val Thr Asp Ser  
 130 135 140  
 Thr Thr Gly Arg Leu Cys Met Leu Val Asp His Glu Tyr Lys Tyr Pro  
 145 150 155 160  
 Tyr Val Leu Gly Gln Gly Gln Asp Thr Leu Ala Pro Glu Leu Pro Ile  
 165 170 175  
 Trp Val Tyr Phe Pro Pro Gln Tyr Ala Tyr Leu Thr Val Gly Glu Val  
 180 185 190  
 Asn Thr Gln Gly Ile Ser Gly Asp Ser Lys Lys Leu Ala Ser Glu Glu  
 195 200 205  
 Ser Ala Phe Tyr Val Leu Glu His Ser Ser Phe Glu Leu Leu Gly Thr  
 210 215 220  
 Gly Gly Ser Ala Thr Met Ser Tyr Lys Phe Pro Ala Val Pro Pro Glu  
 225 230 235 240  
 Asn Leu Glu Gly Cys Ser Gln His Phe Tyr Glu Met Tyr Asn Pro Leu  
 245 250 255  
 Tyr Gly Ser Arg Leu Gly Val Pro Asp Thr Leu Gly Gly Asp Pro Lys  
 260 265 270  
 Phe Arg Ser Leu Thr His Glu Asp His Ala Ile Gln Pro Gln Asn Phe  
 275 280 285  
 Met Pro Gly Pro Leu Ile Asn Ser Val Ser Thr Lys Glu Gly Asp Asn  
 290 295 300  
 Ser Asn Thr Gly Ala Gly Lys Ala Leu Thr Gly Leu Ser Thr Gly Thr  
 305 310 315 320  
 Ser Gln Asn Thr Arg Ile Ser Leu Arg Pro Gly Pro Val Ser Gln Pro  
 325 330 335  
 Tyr His His Trp Asp Thr Asp Lys Tyr Val Thr Gly Ile Asn Ala Ile  
 340 345 350  
 Ser His Gly Gln Thr Thr Tyr Gly Asn Ala Glu Asp Lys Glu Tyr Gln  
 355 360 365  
 Gln Gly Val Gly Arg Phe Pro Asn Glu Lys Glu Gln Leu Lys Gln Leu  
 370 375 380  
 Gln Gly Leu Asn Met His Thr Tyr Phe Pro Asn Lys Gly Thr Gln Gln  
 385 390 395 400  
 Tyr Thr Asp Gln Ile Glu Arg Pro Leu Met Val Gly Ser Val Trp Asn  
 405 410 415

Arg Arg Ala Leu His Tyr Glu Ser Gln Leu Trp Ser Lys Ile Pro Asn  
 420 425 430  
 Leu Asp Asp Ser Phe Lys Thr Gln Phe Ala Ala Leu Gly Gly Trp Gly  
 435 440 445  
 Leu His Gln Pro Pro Pro Gln Ile Phe Leu Lys Ile Leu Pro Gln Ser  
 450 455 460  
 Gly Pro Ile Gly Gly Ile Lys Ser Met Gly Ile Thr Thr Leu Val Gln  
 465 470 475 480  
 Tyr Ala Val Gly Ile Met Thr Val Thr Met Thr Phe Lys Leu Gly Pro  
 485 490 495  
 Arg Lys Ala Thr Gly Arg Trp Asn Pro Gln Pro Gly Val Tyr Pro Pro  
 500 505 510  
 His Ala Ala Gly His Leu Pro Tyr Val Leu Tyr Asp Pro Thr Ala Thr  
 515 520 525  
 Asp Ala Lys Gln His His Arg His Gly Tyr Glu Lys Pro Glu Glu Leu  
 530 535 540  
 Trp Thr Ala Lys Ser Arg Val His Pro Leu  
 545 550  
 <210> 93  
 <211> 396  
 <212> DNA  
 <213> erythrovirus  
 <400> 93  
 cct tta aat tgg gac ctc gaa agg cta ctg gaa ggt gga atc ccc agc 48  
 Pro Leu Asn Trp Asp Leu Glu Arg Leu Leu Glu Gly Gly Ile Pro Ser  
 555 560 565 570  
 ctg gcg ttt atc ctc ctc atg cag ctg gtc att tac cat atg tac tgt 96  
 Leu Ala Phe Ile Leu Leu Met Gln Leu Val Ile Tyr His Met Tyr Cys  
 575 580 585  
 atg acc cca cag cta cag atg caa agc aac acc aca gac acg gat atg 144  
 Met Thr Pro Gln Leu Gln Met Gln Ser Asn Thr Thr Asp Thr Asp Met  
 590 595 600  
 aaa agc ctg aag aat tgt gga ctg cca aaa gcc gtg tgc acc cat tgt 192  
 Lys Ser Leu Lys Asn Cys Gly Leu Pro Lys Ala Val Cys Thr His Cys  
 605 610 615  
 aaa cat tcc cca ccg tgt cct cag cca gga acc gtc acc cac cgc cca 240  
 Lys His Ser Pro Pro Cys Pro Gln Pro Gly Thr Val Thr His Arg Pro  
 620 625 630  
 cct gtg ccg ccc aga tta tat gtg ccc cct cca ata ccc cgt agg caa 288  
 Pro Val Pro Pro Arg Leu Tyr Val Pro Pro Pro Ile Pro Arg Arg Gln  
 635 640 645 650



cca tct ata aaa gat aca gac gct gta gaa tat aaa tta tta act aga 336  
 Pro Ser Ile Lys Asp Thr Asp Ala Val Glu Tyr Lys Leu Leu Thr Arg  
 655 660 665

tat gaa caa cat gta att aga atg cta aga tta tgt aat atg tac aca 384  
 Tyr Glu Gln His Val Ile Arg Met Leu Arg Leu Cys Asn Met Tyr Thr  
 670 675 680

agt ttg gaa aaa 396  
 Ser Leu Glu Lys  
 685

<210> 94  
 <211> 132  
 <212> PRT  
 <213> erythrovirus

<400> 94  
 Pro Leu Asn Trp Asp Leu Glu Arg Leu Leu Glu Gly Gly Ile Pro Ser  
 1 5 10 15

Leu Ala Phe Ile Leu Leu Met Gln Leu Val Ile Tyr His Met Tyr Cys  
 20 25 30

Met Thr Pro Gln Leu Gln Met Gln Ser Asn Thr Thr Asp Thr Asp Met  
 35 40 45

Lys Ser Leu Lys Asn Cys Gly Leu Pro Lys Ala Val Cys Thr His Cys  
 50 55 60

Lys His Ser Pro Pro Cys Pro Gln Pro Gly Thr Val Thr His Arg Pro  
 65 70 75 80

Pro Val Pro Pro Arg Leu Tyr Val Pro Pro Pro Ile Pro Arg Arg Gln  
 85 90 95

Pro Ser Ile Lys Asp Thr Asp Ala Val Glu Tyr Lys Leu Leu Thr Arg  
 100 105 110

Tyr Glu Gln His Val Ile Arg Met Leu Arg Leu Cys Asn Met Tyr Thr  
 115 120 125

Ser Leu Glu Lys  
 130

<210> 95  
 <211> 40  
 <212> PRT  
 <213> erythrovirus

<400> 95  
 Met Ser Lys Thr Thr Asn Lys Trp Trp Glu Ser Ser Asp Lys Phe Ala  
 1 5 10 15

Gln Asp Val Tyr Lys Gln Phe Val Gln Phe Tyr Glu Lys Ala Thr Gly  
 20 25 30

Thr Asp Leu Glu Leu Ile Gln Ile  
 35 40

<210> 96  
 <211> 21  
 <212> PRT  
 <213> erythrovirus

<400> 96  
 Ser Leu Phe Asp Leu Val Ala Arg Ile Lys Ser Asn Leu Lys Asn Ser  
 1 5 10 15  
 Pro Asp Leu Tyr Ser  
 20

<210> 97  
 <211> 24  
 <212> PRT  
 <213> erythrovirus

<400> 97  
 Leu Ser Asp His Pro His Ala Leu Ser Ser Ser Asn Ser Ser Ala Glu  
 1 5 10 15  
 Pro Arg Gly Glu Asn Ala Val Leu  
 20

<210> 98  
 <211> 21  
 <212> PRT  
 <213> erythrovirus

<400> 98  
 Glu Asp Leu His Lys Pro Gly Gln Val Ser Ile Gln Leu Pro Gly Thr  
 1 5 10 15  
 Asn Tyr Val Gly Pro  
 20

<210> 99  
 <211> 22  
 <212> PRT  
 <213> erythrovirus

<400> 99  
 Gly Asn Glu Leu Gln Ala Gly Pro Pro Gln Asn Ala Val Asp Ser Ala  
 1 5 10 15  
 Ala Arg Ile His Asp Phe  
 20

<210> 100  
 <211> 21  
 <212> PRT  
 <213> erythrovirus

<400> 100  
 Ile Lys Asn Glu Thr Gly Phe Gln Ala Gln Ala Val Lys Asp Tyr Phe  
 1 5 10 15

Thr Leu Lys Gly Ala  
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